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Cultural Criteria for the Distinction of Wood-Destroying Fungi.

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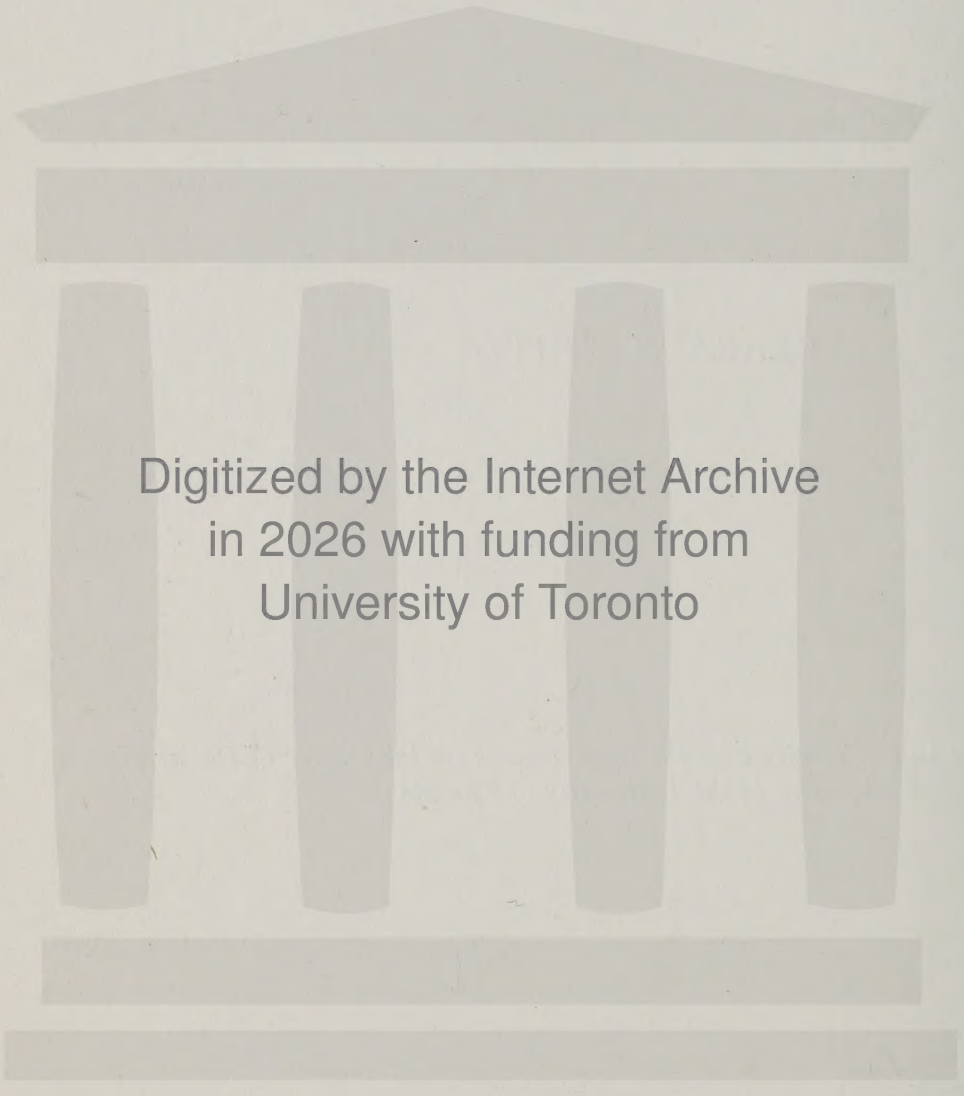
CLARA W. FRITZ

A thesis submitted in conformity with the requirements for the Degree of Doctor of Philosophy in the University of Toronto.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Cultural Criteria for the Distinction of Wood-Destroying Fungi

By CLARA W. FRITZ

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1. INTRODUCTION

In 1874 Hartig established the fungous nature of timber disease and turned the eyes of scientific men upon a new and fruitful field of research. The necessity of forest conservation, long felt in Europe and ever becoming more pressing in America, has combined an economic with the scientific interest incited; and the work of an ever increasing list of investigators, including Ward, von Schrenk, Spaulding, Atkinson, Buller, Falck, Faull, White, Zeller, Long, etc., attests recognition of the value of the work involved and the varied nature of the problems presented.

Wood-rotting fungi are of widespread occurrence in our forests, where the results of their destructive activity are now but too well known to the lumberman. Estimates of the destruction wrought are

unattainable, but from northern Ontario sections are reported (22) where 75% of some species of the standing timber has been found infected. As far as our present knowledge goes the principal causal agents of decay belong to the polypores, the fruit bodies of which, commonly known as punks or conchs, are abundant on the trees of forest, wood-lot, and park, as well as upon exposed structural timber. Their presence informs the initiated of an advanced stage of disorganization of the tissues of the wood upon which they appear.

These wood-rotting fungi show specific differences in their choice of substrata. Among the organisms studied *Polystictus abietinus* (Dicks.) Fr. has been isolated from sapwood only; *Polyporus schweinitzii* Fr. is thought to confine itself to the heartwood. Many species, however, including *Poria subacida* Peck and *Fomes pinicola* (Sw.) Cooke prey upon both indiscriminately. The majority can live on fallen timber or dead portions of standing trees, and many, e.g., *Fomes applanatus* (Pers.) Wallr. (55) and *Polystictus versicolor* (L.) Fr. are reported as capable of development as either parasites or saprophytes. In such cases débris upon the forest floor constitutes breeding centres of infection dangerous to healthy trees in the neighbourhood. A few fungi are limited in their range by their dependence upon a certain host. Among these *Polyporus betulinus* (Bull.) Fr. grows on the birch only. Others develop on a certain class of hosts. Thus *Trametes pini* Fr. has been found confined to conifers. But a few, including *Polyporus sulphureus* Fr., seem unlimited in their potentiality for wood destruction.

The importance of the organisms under consideration is easily conceived. In spite, however, of their abundance and of the widespread destruction which they cause, the surprising fact remains that the specific agents at work in the majority of rotted trees are at present unknown even to those most expert in forest pathology. Two features have in the past furnished the only criteria known, upon which identification could be based; the purpose of the present investigation is to add a third, the peculiarities of which, it is hoped, will supplement those already in use and thus provide an adequate method of determining the causal organisms of wood-rots.

Until within the last few years identification has been based entirely upon the proximity of sporophores and the characteristics displayed by the wood attacked. The former is open to the criticism that the presence of a fruit body on the exterior of a rotted tree is no guarantee that such a fruit body is a production of the mycelium of the active agent of destruction in the interior. In regard to the latter, as the number of fungi investigated increases, the suspicion arises

that different forms may act in certain cases similarly upon their substrata. It is here proposed to employ as diagnostic characteristics the peculiarities of the vegetative organs when grown massed in pure culture. Owing to the microscopic dimensions of the individual elements of these organs, and to their usual diffuse growth under natural conditions, these peculiarities are not available in the field. Under laboratory conditions, however, luxuriant growth is readily obtained; and a study of the species here recorded has revealed easily recognized distinctions both macroscopic and microscopic.

2. Historical Review

(a) *Organisms under natural conditions*

The organisms considered in the present investigation belong for the most part to the polypores, many of the large fruit bodies of which have doubtless been known to man since pre-historic times. The recognition of their economic importance, on the other hand, is of recent date. Few references to the polypores are found in classical literature. The chief interest of the ancients in regard to fungi seems rather to have been in relation to gastronomy, as witnessed by the first system of classification, that of Dioscorides, which separates the then known species into edible and poisonous forms. The comments of classical authors have, therefore, been chiefly upon fleshy fruits. Two woody species, however, early received recognition in the practice of medicine. Of these the first is referred to by Hippocrates in the fifth century B.C., when he advised the use of tinder made from a fungus, probably *Fomes fomentarius* Gill., in cauterization for the cure of certain ailments. The second, *Fomes officinalis* Fr., commercially known as agaricum, was according to Dioscorides a veritable panacea; but his ignorance of its nature is evidenced by his statement that: "Some people say that it is the root of a plant, others that it is produced like fungi in the trunks of trees that have become rotten." (12)

Accurate study and classification of the fruit bodies dates back little more than a hundred years. Linnaeus, with the introduction of the binomial system of nomenclature in 1753, listed only twelve species of pored fungi and included all, both fleshy and woody forms, in the one genus Boletus. By 1801 the number of recognized forms had greatly increased and Persoon (38) describes ninety-eight species, grouped into two genera according to pore forms. Fries' "Systema Mycologicum" 1821 has been adopted as the starting-point for nomenclature; and many investigators, including such men as Gillet,

Quélet, Patouillard, Karsten, Hennings and Murrill, have by additions, regrouping and reclassification increased and rearranged the known forms until the recent works of the last-named author include sixty genera exclusive of resupinate forms. Each of these plays some part in the reduction of woody substances to their simpler components, processes beneficial to the forester or the reverse, according to the value of the substrata attacked.

The publication by T. Hartig of "Abhandlung über die Verwandlung der polycotyledonischen Pflanzenzelle in Pilz-und Schwammgebilde und die daraus hervorgehende sogenannte 'Fäulniss des Holzes'" in 1833 presented the first scientific study of wood rots. Hartig recognized a connection between the presence of fungous hyphae and the decomposed condition of decayed wood tissues, but in accounting for the mycelium he resorted to the theory of spontaneous generation. He stated that, owing to age or some external factor, the elements of the wood were dissociated. These particles then reunited and produced filaments endowed with life and the power of growth, the continued formation and development of which took place at the expense of surrounding tissues.

Schacht (43) in 1863 in an investigation on the changes wrought by fungous hyphae in dead plant cells included an account of the action of several wood-destroying forms. Among others he noted the fungous nature of the rots of oak, horse-chestnut, spruce, and beech, and discussed the solution of cell walls and contents in contact with advancing hyphae. Secondary spores were also observed, but in the absence of organized fruit bodies he confessed himself unable to account for the origin of the invading organisms. Germination tests with spores obtained from diseased wood of *Dracena draco* led to no results; and attempts to culture the mycelium in naturally infected blocks to fructification in moist chambers also proved unsuccessful.

Willkomm in his "Die mikroskopischen Feinde des Waldes" (1866) reported studies upon wood decay, and recognized its fungous origin; but his ideas of the developmental stages were fanciful and not founded upon direct observation. His work, therefore, adds no important contribution to a knowledge of the subject.

The theories and suggestions of earlier investigators led up to the fundamental work of R. Hartig, who in 1874 published his earliest treatise, "Wichtige Krankheiten der Waldbäume." In this he recorded the proof of his theory of the parasitism of the Hymenomycetes by means of successful infection of the pine by *Trametes pini*. Infection and culture methods did not, however, constitute his manner of attacking the problem; and his most extensive publication on

wood-destroying fungi (25) embodies an exhaustive study of the histological changes in different kinds of wood due to the damaging effect of fungous organisms. In this he recorded the results of the action of seven forms upon coniferous wood and of six others upon the wood of the oak. In each case, after a careful anatomical study of the sporophore of the fungus under consideration, he described with minute detail the progress of decay as noted both macroscopically and microscopically; and he emphasized as his fundamental principle the constancy and uniqueness of the type of rot produced by each species investigated.

Mayr (33) in 1884 studied the action of two parasites of the birch, *Polyporus betulinus* Bull. and *Polyporus laevigatus* Fr., upon the wood of their host. In this research he followed in the footsteps of R. Hartig, and discussed the physical and microchemical effects of the invading fungi. In addition he recorded successful transmission of disease to a sound birch trunk by means of the insertion of an infected plug.

Later work on wood-destroying fungi has for the most part combined anatomical with cultural studies, and will be taken up in a review considered from the standpoint of the latter. Before passing to this, however, mention must be made of a series of papers by von Schrenk, who from a practical point of view has discussed their economic significance, methods of infection and development, and the possibilities of prophylactic and therapeutic treatment. He has reported with some detail the distribution, hosts, habits and specific methods of attack of a large number of species, which prey upon both coniferous and deciduous wood.

(b) *Cultural studies.*

The pursuit of aims quite varied in nature has stimulated the work of different men in their cultural studies of fungi. Early investigators were especially interested in the successive stages of their life histories with a view to knowledge of group relationships and correct classification. The first students of the results of fungous action upon woody tissues obtained their material from naturally infected trees; but the possibility of the presence of secondary organisms was soon recognized, and the necessity of confirmation by comparison with wood decayed under controlled conditions in pure culture felt. Secondary spore forms, their viability and relative importance in fungous dissemination, has formed the basis of valuable work. Physiological studies, including enzyme action and reactions to environmental conditions, have received attention. Lastly references are

found to the identification of organisms by cultural methods, either by the production of sporophores on naturally infected blocks, or by a study of specific characteristics exhibited by vegetative growth in pure culture.

The classical researches of Brefeld (5-7) covering many years and involving observations on over two hundred species of Basidiomycetes had for their aim the study of sporophore development. In theory his method was ideal: to start with a single spore and carry through under direct observation to normal fructification. This he achieved in several agarics and from his observations decided that reproduction in the Autobasidiomycetes is asexual. Brefeld successfully germinated the spores of over two hundred species and made extensive observations on mycelial characteristics.

In attempting to test certain results of Brefeld with regard to food requirements of *Nyctalis lycoperdioides* and the conditions favoring sporophore production by *Marasmius oleae*, Costantin (14) cultured to fructification both organisms, and noted variations induced by growth upon different substrata. Three years later Costantin and Matruchot (15) exhibited pure cultures of *Collybia velutipes* containing normal, mature fruit bodies. These cultures originated from spores, which after germination were transferred to blocks of sterilized wood.

Ward (52) made a cultural study of *Stereum hirsutum* with a view to isolating and testing the enzymes present, but the production of sporophores led him to a closer examination of its life history. He cultured the fungus from spore to spore; tested its growth on horse-chestnut, pine, willow, and oak; compared its power of attacking sapwood and heartwood; examined in detail its action upon the wood; and made a careful anatomical study of the sporophores obtained.

The next year Biffin (3) published the results of a similar study of the action and development of *Collybia velutipes*; and three years later he followed this up with an account of his observations upon the growth in pure culture of *Bulgaria polymorpha* (4).

In 1902 Falck (17) recorded the successful culture to maturity of three xylophilous agarics; and later (19) described fruit body formation in pure culture on agar, bread, wood, etc., of eight wood-destroying fungi, including *Polyporus vaporarius*, *P. betulinus*, and *Coniophora cerebella*.

Spaulding (50) following the methods employed by earlier investigators traced the development of *Schizophyllum commune* from

spore to spore, and recorded also the production of abnormal fruit bodies of *Lenzites sepiaria* in culture.

In the same year Buller (9) reported the successful identification by cultural methods of the organism causing the rotting of wooden paving blocks in the city of Birmingham. Infected blocks from the city streets were placed in moist chambers in the laboratory, and in the course of a few weeks normal sporophores of *Lentinus lepideus* developed. On wood from the first locality from which collections were made this was the only organism obtained, and it was, therefore, concluded to be the active agent of destruction. The following year Buller (10) published a comprehensive study of *Polyporus squamosus*, including its distribution, structure, spore germination, action on wood, and production of enzymes. Buller confined his cultural work to spore germination studies; but our knowledge of this fungus was enlarged a few years later by the work of Price (39) who, starting with the spore, carried his cultures through to the production of sporophores, which, however, proved sterile. He included observations upon mycelial characteristics and the decay of wood in pure culture.

We are indebted to Lyman (32) for the most extensive researches yet published upon polymorphism among the Hymenomycetes. He succeeded in culturing from spores about seventy-five forms and has given an account of his observations upon twenty-eight, including species of the Thelephoraceae, Hydnaceae, and Polyporaceae. Of these, five possess oidia, nine produce chlamydospores, and six, which are dealt with in detail, have secondary methods of reproduction of especial importance.

Bayliss (2) carried out an investigation upon *Polystictus versicolor*, and succeeded in obtaining mature sporophores, although not until the infected blocks were removed from the laboratory and placed under more natural conditions in the open.

Rumbold (42) has discussed the mycelial characteristics shown in culture of a large number of wood-destroying forms, including of the organisms here considered *Fomes fomentarius*, *F. igniarius*, and *F. pinicola*.

Zeller (56) has reported a careful study of *Lenzites sepiaria* with respect to its cultural characteristics and factors governing its growth and metabolism; and has given particular attention to the enzymes present and their relation to wood decay. The following year he extended this by an investigation of the physical properties of wood in relation to decay induced by the same organism (57). Three species

of yellow pine differing in physical properties were used as substrata for growth of the fungus, and a comparative study of its action on these carried out.

Rhoads (41) has given us a lengthy paper upon the biology of *Polyporus pargamenus*, in which he considers its distribution, anatomy, action upon wood and cultural characteristics.

In a very complete study of the biology of *Fomes applanatus*, White (55) reported the observation of its entire life history under controlled conditions. His cultures were started from spores, sporophore tissue, and decayed wood; and produced practically identical mats with definite and easily recognized characteristics.

Hiley (30) in an account of diseases of the larch has recorded similar success in the culture of *Fomes annosus*. Fructifications in both cases, however, were obtained on natural media only.

The constancy of the mycelial peculiarities of wood-destroying fungi in pure culture was recognized by Brefeld, but his extensive observations led to no system of identification. Möller (35) in his study of fungi responsible for dry rot of building timber pointed out the value of vegetative peculiarities in differentiating between the organisms concerned. Falck (18) collaborating in this work by a very thorough and detailed research upon four species of Lenzites emphasized the diagnostic value of mycelial characteristics, both morphological and physiological. Faull (20) considering the wood-destroying fungus *Fomes officinalis* makes a comparison between tissue cultures obtained from that organism and those from *Polyporus sulphureus*, and notes the ease with which the two may be separated by the characteristics displayed. Long and Harsch (31) were the first to devote special attention to the possibilities of building up a set of diagnostic characteristics of wood-destroying fungi by cultural methods. In an interesting paper they have detailed the peculiarities of several forms, and emphasized their value in the determination of the causal agents of unknown rots. They also met with marked success in the production of sporophores on artificial media, reporting the development of six hundred and forty fruit bodies, representative of forty-two species included among the Agaricaceae, Polyporaceae, Thelephoraceae, and Tremellaceae. A paper by Snell (49) deals with five fungi of importance in the decay of building timber. He considers in particular factors favoring their development and dissemination, but enumerates also the peculiarities, both macroscopic and microscopic, which make possible the determination of each when grown in pure culture upon artificial media.

3. List of Species Discussed and Diagnostic Characteristics Considered

The present investigation was undertaken with the object of obtaining, if possible, a set of cultural characteristics, which would serve for the identification in the laboratory of wood-destroying fungi; and has been directed in particular toward the determination of heart rots of *Abies balsamea*, which are extremely abundant and destructive in the forests of Ontario and Quebec. I am indebted to Dr. J. H. Faull for most of the material upon which the study is based. It has been collected from various localities in both provinces, and is preserved in the Herbarium of the University of Toronto. The following seventeen organisms have been considered and with one exception the peculiarities of each found constant and unique:

1. *Fomes applanatus* (Pers.) Wallr.
2. *Fomes fomentarius* (L.) Gill.
3. *Fomes igniarius* (L.) Gill.
4. *Fomes pinicola* (Sw.) Cooke.
5. *Fomes roseus* (Alb. & Schw.) Cooke.
6. *Polyporus borealis* Fr.
7. *Polyporus schweinitzii* Fr.
8. *Polyporus sulphureus* Fr.
9. *Polystictus abietinus* (Dicks.) Fr.
10. *Polystictus versicolor* (L.) Fr.
11. *Trametes pini* Fr.

Rots of *Abies balsamea*:

12. Type A. (1) *Polyporus balsameus* Peck.
13. (2) *Fungus* sp.
14. Type B. (1) *Poria subacida* Peck.
15. (2) *Fungus* sp.
16. (3) *Fungus* sp.
17. Type C. (1) *Fungus* sp.

All have been cultured from a number of sources detailed in each case in the discussion of individual species which follows.

The peculiarities judged to be of diagnostic value are macroscopic and microscopic. The former include texture, color, rate of growth, manner of advance over the agar surface; the latter: types of hyphae, color, method and frequency of branching, septation, occurrence and type of clamp connections, and production of secondary spore forms. Some organisms have also a characteristic odor.

4. Conditions of Growth

A. *Type of culture.*

Cultures used as standards have been obtained from sporophore tissue, small inocula being removed under aseptic conditions from the interior of fresh fruit bodies and half buried in the agar upon which they were to be grown. Cultures for comparison or identification were obtained in a similar manner from fresh specimens of rotted wood. Sporophore and decayed wood cultures of each fungus have been found practically identical; and no difficulty is experienced in determining the identity of an organism isolated from rotted wood provided a corresponding sporophore culture is at hand.

Cultures were grown in tubes of different sizes, in erlenmeyer flasks, and in petri dishes. For solid media the first proved the most practical from the point of view of economy of space and insurance of continued purity of growth. Tubes 6″ x 0.9″ were finally selected for comparative work, since they furnished an agar slant surface of sufficient area to provide a clear view of the variations exhibited. For each culture fifteen c.c. of medium were used and the tubes were slanted to the same degree in order to provide surfaces of uniform area.

In the comparison and selection of mycelial peculiarities cultures of the same age were used; and the inocula from which they were started were taken from the actively growing portions of young cultures in order that initial growth conditions might be as uniform as possible.

B. *Media used.*

(*a*) Composition.

(*a*) Solid media.

(1) Potato-dextrose agar. 400 g. sliced potatoes and 1 l. distilled water were steamed in an autoclave 30 min. at 15 lbs. pressure and strained. The potato decoction thus obtained was used to replace the unabsorbed water drained from 25 g. shredded agar, which had been soaked overnight in 1 l. distilled water. The agar was then melted by steaming in an autoclave 20 min. at 15 lbs. pressure, and on removal 25 g. dextrose were added before tubing. To prevent prolonged decomposition of the sugar from the action of heat, the autoclave was heated to steaming before the tubes were placed in it for sterilization. They were then sterilized 10 min. at 15 lbs. pressure.

(2) Vegetable agars. These were made according to the method used in medium (1), without the addition of dextrose and using the

following weights per litre: seed corn 80 g.; carrot 500 g.; bean 400 g.; pea 400 g.; beet 500 g.; oatmeal 80 g.; celery 600 g.

(3) Czapek's synthetic agar.

0.5 g. magnesium sulphate.
1.0 g. mono-potassium phosphate.
0.5 g. potassium chloride.
0.01 g. ferrous sulphate.
2.0 g. sodium nitrate.
30.0 g. dextrose.
25.0 g. agar.
1 l. distilled water.

The chemicals, with the exception of dextrose, were dissolved in the water and added to the agar. This was then melted as in other media, the dextrose added before tubing, and the tubes sterilized as described above.

(4) Czapek's synthetic agar (modified). In this medium the sodium nitrate of medium (3) was replaced by 20 g. peptone, which was dissolved with the dextrose in 200 c.c. of the water used and added just before tubing. The preparation was otherwise similar to that of medium (3).

(5) Currie's synthetic agar (modified).

1.00 g. mono-potassium phosphate.
0.25 g. magnesium sulphate.
0.05 g. ferrous sulphate.
20.00 g. peptone.
30.00 g. dextrose.
25.00 g. agar.
10 c.c. N/10 hydrochloric acid.
1 l. distilled water.

This was prepared by the method used in medium (4).

(6) Malt.

25. g. extract of malt.
25. g. agar.
1 l. distilled water.

The agar was soaked overnight in 1 litre distilled water, and the unabsorbed water replaced by an equal amount containing the malt extract. It was then steamed 20 min. at 15 lbs. pressure, strained, tubed, and sterilized 10 min. at 15 lbs. pressure.

(β) Liquid media.

(1) Czapek's synthetic medium.

(2) Czapek's synthetic medium (modified as above).

(3) Malt.

These three media were prepared as above with the omission of agar.

(4) Duggar's synthetic medium.

10.00 g. potassium nitrate.
0.50 g. mono-potassium phosphate.
2.50 g. magnesium sulphate.
0.02 g. ferric chloride.
40.00 g. dextrose.
1 l. distilled water.

The nutrients were combined and sterilized as in other synthetic media.

(*b*) Acidity.

In considering the necessary exactness of standardization of conditions for comparative work the question arose as to the effect of variations in the acidity of the culture medium. The growth of four organisms—*Fomes applanatus*, *F. fomentarius*, *F. pinicola* and *Polystictus versicolor*—was tested, and indicated such a range of acid tolerance that any adjustment of the natural media, in which under standard conditions of preparation the variation is slight, was deemed inessential; and in spite of the recognized imperfections of the methods of standardization employed in the test series, the conclusion has proved warranted, since each species has yielded a constant growth upon media prepared as described.

The organisms tested were grown on potato-dextrose agar standardized by the titration method as recommended by Harshberger (24) in his suggestions for laboratory technique. The agar prepared for tubing was divided into equal volumes. An aliquot portion was then titrated with $\frac{N}{20}$ NaOH using phenolphthalein as indicator, and the separate volumes adjusted to the required acidities by addition of the calculated amounts of $\frac{N}{NaOH}$ and $\frac{N}{HCl}$. The subsequent changes due to sterilization were not determined.

It is to be noted that the ranges of acid tolerance shown by individual organisms on different vegetable media standardized by the above method need not coincide, since different buffer reactions may provide very unequal amounts of free acid even after such standardization. Duggar, Severy, and Schmitz (16) in a study of the growth of certain fungi in plant decoctions remark upon the great difference in H-ion concentration between corn-meal and potato decoctions after

standardization to +15 by the above method, and note the corresponding differences in growth of the fungi tested. Corn meal decoction had a pH value of +2, potato decoction a value of +4.

Using, however, the standards obtaining in the experiments under consideration the four organisms showed considerable difference in their reaction to changes in acidity. *Fomes applanatus* showed the most decided tolerance of high alkalinity. The exact limit was not ascertained, but growth was obtained at −100. *Polystictus versicolor* was slightly more limited in its range; *Fomes fomentarius* was even less adaptable; and *Fomes pinicola* showed a distinct preference for an acid medium, and grew in no culture where the determined acidity was lower than +5. On the acid side of the scale the limit of growth for the first three organisms was the same: they did not grow beyond +30. In *Fomes pinicola* growth was not inhibited at +50. The natural potato-dextrose medium tested for many series was found to fall within the range common to all. This agar unmodified has been found most satisfactory for all other organisms tested, and growth upon it has been taken as the main standard of classification for the forms compared.

C. *Light and Temperature.*

Long and Harsch (31) found it advantageous to expose their cultures to the sunlight since the color characteristics were thereby accentuated and the mycelial growth retarded. They state: "The differentiation of the characters of the mycelium produced both as to texture and color of the aerial mycelium, is very much more marked when the cultures are grown in the presence of light at ordinary room temperatures than when grown in incubators at the optimum and constant temperature for the mycelial growth of the fungus under consideration;" and again: "Cultures when grown in darkness and at a more or less constant and high temperature overrun very rapidly the surface of the agar in the tube, thus obscuring the real growth of the fungus as observed in the cultures subject to daylight conditions." Since the aim of the research in hand is the differentiation of species by morphological characteristics rather than a comparative study of optimum conditions for individual organisms the necessity of the 'more or less constant and high temperature' has not been felt, and a retardation of growth such as Long and Harsch suggest seems not unfavorable to the object in view. In consideration, however, of the desirability of making possible repetition of the work for purposes of comparison, it seemed advisable to test the effect of the amount of

variation comprehended in the terms 'daylight' and 'ordinary room temperature.'

Light. Each of the seventeen fungi under consideration when grown in darkness produced a mat of distinct type, and where pigment was developed a characteristic coloration. In no case tested was a mat typically pale in the dark found to produce marked coloration under the influence of light. Of the pigmented forms *Fomes igniarius, F. fomentarius, F. roseus, F. pinicola, Polyporus schweinitzii* and *Trametes pini* were tested in darkness, diffuse light and in a position receiving about three hours' direct sunlight. Growth in the last-named position was in some cases greatly retarded as may be noted in Figs. 15 and 20. It was, however, characteristic and the hyphae, although somewhat unhealthy and aborted in appearance, branched out when removed to either of the other positions and produced the normal growth ordinarily obtained there. Marked variation in type may be caused by the influence of diffuse light as is shown in Figs. 21-24. The cultures there shown depict the development of *Polyporus schweinitzii* on potato-dextrose (Figs. 21 and 23) and malt (Figs. 22 and 24) agars in diffuse light and darkness. On both media light causes a limitation of growth and an intensification of color, but neither in this case of extreme variation nor in others where changes are less marked does elimination of light effect any confusion of organisms due to consequent variations in texture or coloration. The characteristics of Figs. 23 and 24 are quite as typical of *Polyporus schweinitzii* as is the more compact growth of Figs. 21 and 22.

The species tested were grown on potato-dextrose agar, and a set of four tubes of each was maintained in each of the positions chosen. Each set proved sufficiently uniform and unique to characterize any organism, but the mats of the first two positions were more healthy than those receiving direct sunlight.

Fomes igniarius (Figs. 14 and 15). Sunlight as received by the tubes under consideration effected the decided limitation of growth and deepening of color illustrated in Fig. 15. This mat, showing the results of two weeks' growth, had a felty surface of an even Isabella color shaded to cream buff at the edges. In darkness and diffuse light development was very similar, no noticeable difference occurring in the amount of growth and but slight variation in depth of color. *Fomes fomentarius* (Figs. 19 and 20). The reaction to light was very similar to that exhibited by *Fomes igniarius*. Darkness and diffuse light effected the development of mats of the same type, but two weeks' growth in the former produced a mat of pinkish cinnamon to light pinkish cinnamon with a considerable border of

white, which was rather markedly greater in area than the more deeply toned sayal brown to cinnamon mat which developed in the latter. Sunlight limited both growth and pigmentation (Fig 20.) An abrupt white border surrounded the sayal brown to cinnamon circle in the vicinity of the inoculum. *Trametes pini* resembled in its reaction to light the last two organisms. The coloring and texture in darkness and diffuse light were not markedly different: white, tinted in the older growth with honey and pinard yellow; but growth in darkness was slightly more profuse than in the second position. Sunlight induced a limitation of growth and a hardening of the mat. Aerial coloration was as above, but a thin submerged layer was differentiated by its russet and sulphine yellow pigmentation.

Fomes roseus. In the position in which this fungus was first tested direct sunlight was received about one hour and a half each day, and the resulting mats from darkness, diffuse light, and sunlight seemed to indicate an indifference to light conditions. In all three there were produced the almost white, downy-flecked mats, characteristic of the organism. But cultures placed in the position receiving full three hours of sunlight showed a distinct response to the increased light. Spread of the mycelium was distinctly retarded, and the color deepened. At the end of two weeks the agar slant was about half covered. In the older growth there was produced a somewhat hardened circle with felty surface and fawn to pale ochraceous salmon coloration. Growth towards the outer limits was a thin, white film.

Polyporus schweinitzii. As indicated above, the mycelium of this fungus has been found to exhibit greater sensitivity to the presence of light than any of the other organisms tested. Direct sunlight proved almost inhibitive. In one week the entire growth consisted of a dull submerged circle, covered by a sodden, appressed, colorless aerial layer. In two weeks the surface growth was thin, filmy, and dull, with a faintly velvety appearance. Diffuse light permitted the development of a profuse and brilliantly colored mat (Fig. 21). At the end of a week an inner velvety circle of aniline to wax yellow was evident, and this extended, thickened, and deepened in tint to pyrite, sulphine and strontian yellow. In comparison with this thick growth and vivid coloration development in the dark was inconspicuous. The initial furry-filmy spread of pale cream color extended rapidly, and thickened to a close filmy-downy mat, which toward the top of the agar slants formed thick wads, often of sufficient size to plug the tubes. Stalks, suggesting the beginning of fructification, also developed on the upper part of the mat. These thicker areas were more deeply

colored than the surrounding film, but in no way matched development in the light. The microscopic differences in hyphal development and arrangement explain the macroscopic distinctions. In the light, regular firm-walled hyphae were stranded together to form tufts, which growing massed together and perpendicular to the surface produced a velvety appearance. They were deeply pigmented and without spores. In the dark, the aerial mat presented a loose tangle of irregularly, much-branched threads very similar to those of the submerged layer. They were hyaline, or slightly yellow in mass and contained chlamydospores. A few scattered threads were deeply pigmented. The thicker areas contained the firmer walled, pigmented hyphae formed in the velvet of mats from the light. The stalks were formed of a thick core of these, closely intertwined and covered with a velvety layer formed as above described.

Fomes pinicola. The fungus was grown on potato-dextrose, potato, corn, Czapek's synthetic and Currie's synthetic (modified) agars in all three positions. Light to some extent retarded the spread of mycelium and sunlight caused some variation in color, producing on the first named agar a white mat shadowed with vinaceous buff instead of the pale ochraceous salmon of the first two positions. Although never deeply tinted the most decided and general coloration was obtained in the dark and it was most pronounced on the last-named agar, on which the greater part of the mat assumed a coloration of light ochraceous salmon.

The results in all cases indicate the unfavorable action of direct sunlight, the slight retardation of growth, and color accentuation in diffuse light, and the possibility of obtaining distinctive development in the dark. The sensitivity of *Polyporus schweinitzii* proves the necessity of standardizing conditions and the difficulty of providing uniform light intensity makes growth in darkness the more natural choice.

Temperature. Having decided that specific characteristics were in no way masked by growth in darkness the effect of incubation at ordinary room temperatures was tested for several organisms. *Fomes fomentarius, F. igniarius, F. roseus, Lenzites sepiaria, Polyporus schweinitzii,* and *Balsam rot Type B* (*2*) were grown at 22°, 27°, 30° and 35° C. The last temperature, though not impossible, is beyond the usual range. It proved satisfactory for but one species, *Lenzites sepiaria*; in all other cases growth was greatly retarded (Figs. 13, 18 and 48). At the lower temperatures the amount of variation was insufficient to cause any hesitation in the grouping of cultures. Specific differences were noted in the action of heat upon rate of growth and

depth of coloration, but the variation was in no case sufficiently marked to cause confusion of types.

As in the tests upon the action of light the fungi with the exception of *Lenzites sepiaria* were grown in test-tubes in sets of four at each of the temperatures chosen, and exhibited marked regularity in their response to the conditions to which they were subjected. *Lenzites sepiaria* was cultured on upright agar surfaces in erlenmeyer flasks, and in each position cultures were carried in duplicate only.

Fomes igniarius (Figs. 11, 12 and 13). With each rise of temperature up to 30° C. the amount of growth was increased and the color deepened. In all three, however, the type remained constant and the color varied in depth only. At the end of two weeks at 22° C. the growth covered 5 cm. of the slant and was chamois in color with a margin of white (Fig. 11); at 27° C. the mycelium had extended 1 cm. farther up the slant and the color was almost buckthorn brown in the older growth with chamois to white toward the upper limits (Fig. 12); at 30° C. the slants were almost covered and the coloration deep; almost tawny-olive with a cream buff to white border. At 35° C. growth was greatly retarded: represented at the end of two weeks by a small circle, less than 2 cm. in diameter (Fig. 13). This was antique brown with a narrow cream buff border and a downy-velvety surface. Microscopically it was noted that this high temperature caused profuse branching and septation in both pigmented and hyaline threads; and the walls of the former were thinner than those of corresponding hyphae developed at lower temperatures. *Fomes roseus* (Figs. 46, 47 and 48). The response to rise in temperature was very similar to that shown by *Fomes igniarius*. At 22°, 27° and 30° C. there was produced the downy-flecked mat typical of the organism, although at the last-named temperature the flecks had a tendency to thicken and become felty. Growth was increasingly greater in extent with each rise in temperature, and at 30° C. there was a perceptible and general shadowing of pale ochraceous salmon. Growth at 35° C. was compact and as it advanced became radially furrowed. Development was slow and pigmentation deep and general. At the end of two weeks the coloration was a solid avellaneous.

Fomes fomentarius (Figs. 16, 17 and 18). In this species also growth increased with rise in temperature up to 30° C., but unlike the last two fungi pigmentation was adversely affected. At the lowest temperature the cinnamon brown of the older portion shaded through pinkish buff and light pinkish cinnamon toward the upper limit; at 27° C. the last-named colors were the deepest obtained and a considerable portion of the mat toward the top remained white; at

30° C. the coloration was pale: light pinkish cinnamon to white, although at the end of two weeks a slight staining of sayal brown was becoming evident toward the top. A noticeable accumulation of moisture was obtained on the surface, but decreased in amount with increase of heat. This exuded from a deeply pigmented layer at the surface of the agar and was especially abundant at 22° C., where passing up through the mat it lay in large drops on the surface, and down into the agar caused a deep staining. At 30° C. the drops were minute, pale, and inconspicuous. At 35° C. growth was slight. The inoculum became covered with a thin velvet, raw umber in tint and composed of upright, densely pigmented and much septated hyphae, in which the cells were often not more than 6-10 μ in length, and with walls thinner than those of pigmented hyphae at lower temperatures. The slight spread was white, with no underlying pigmented layer and no exudation of moisture.

Polyporus schweinitzii. The best growth was obtained at 27° C., but at 22°, 27° and 30° C. the typical furry-filmy, slightly yellowed mat was developed. At 35° C. no aerial mat was formed. At the end of two weeks there was produced merely a dull, submerged circle, which was gradually spreading, and which when placed at a lower temperature developed into a characteristic filmy mat. *Balsam rot Type B (2).* This reacted as did *Polyporus schweinitzii* giving typical mats at 22°, 27° and 30° C. with growth slightly more profuse at 27° C. In all three, characteristic delicate yellow coloring was present at the end of two weeks. At 35° C. growth was very slight, the mycelium spreading in a delicate, colorless film but a short distance from the scantily covered inoculum.

Lenzites sepiaria grew with great luxuriance at 35° C., which is recorded by Falck (18) as its optimum temperature. In ten days an agar surface of approximately 45 sq. cm. was well covered, while at 22° C. the mycelium had spread over an area not more than one quarter as large. Coloration developed earlier and with greater intensity at 35° C. At the end of ten days the older growth had assumed a warm buff tinting, and this gradually deepened to include chamois, buckthorn and Prout's brown; the agar also was deeply stained. Two weeks' growth in flasks at 22° C. left the mats still white, but later, tints of pale pinkish cinnamon, chamois and honey yellow appeared. A similar coloration was obtained in flasks kept in diffuse light, which testifies to the efficacy of heat rather than light in color intensification in the species. In all flasks dissemination by oidia was evidenced by the slimy growth in the liquid collected at the bottom of the flasks.

Low and high temperatures retard growth, development between 25° and 30° C. proving most satisfactory for five of the six fungi tested. Within the temperature range of the experiments under consideration retardation at the lower limit provided the more healthy growth. Color intensification was effected by increase of heat in the case of *Fomes igniarius, F. roseus* and *Lenzites sepiaria* in as great or even greater degree than by the agency of light; but as in the case of light the organisms varied in their response, coloration in *Polyporus schweinitzii* and *Balsam rot Type B* (*2*) being uninfluenced by temperature and *Fomes fomentarius* showing more delicate tinting toward the upper limits. While the results show that characteristic and easily recognized mats of the fungi tested may be obtained throughout the range of ordinary room temperatures, the somewhat slow growth provided at 22° C. was deemed satisfactory and has been used in the comparative work detailed below.

5. Growth on Liquid Media

The wood-destroying fungi tested grew well on suitable liquid media, but diagnostic characteristics were, on the whole, longer in development, and proved no more striking in appearance than those displayed on a solid substratum. Cultures of the latter type are also more easily stored and handled than those of the former. *Polyporus balsameus, Fomes applanatus, Polyporus sulphureus, Trametes pini* and *Balsam rot Type B* (*2*) were grown on malt, Czapek's synthetic medium, Czapek's synthetic medium (modified) and Duggar's synthetic medium. The cultures were carried in sets of three in erlenmeyer flasks of 200 c.c. capacity, each furnished with 50 c.c. of medium. Inocula were obtained from agar cultures corresponding in nutrient composition to the contents of the flasks inoculated.

Of the four media Czapek's and Duggar's synthetic solutions were unsatisfactory, but good growth was obtained on malt and Czapek's medium containing peptone. Superficial growth, which produces the most distinctive characteristics, developed readily from a floating inoculum, but all grew with greater or less rapidity from a submerged inoculum, and on suitable media produced in time characteristic aerial mats. In a week *Polyporus sulphureus* on malt produced from either submerged or floating inocula its readily distinguished aerial mycelium bearing immature spores. *Fomes applanatus* was slower in the production of an aerial mat from a submerged inoculum, but in the course of three weeks it also was readily identified. At the end of a month the growth of *Trametes pini* and *Polyporus balsameus* from submerged inocula was represented merely by compact, submerged, translucent

balls indistinguishable macroscopically except in the more profuse development of the former species. From a floating inoculum three weeks' growth of *Polyporus balsameus* yielded several small circles probably originating from scattered spores. These were soft and white with a central shadowing of yellowish brown, and the mycelium was without spores. Later, however, spores developed and at the end of two months were abundant in the more deeply browned patches over the surface. In three weeks *Balsam rot Type B* (*2*) had produced its distinctive soft, white aerial mat. No fibre-like threads were developed and even at the end of two months they were scantily represented. After that period also the mycelium remained pure white, without any shadowing of yellow as obtained on solid media.

6. Descriptions of Species Investigated

Conditions of culture. The cultures described below were grown in culture tubes 6″ x 0.9″ in size each containing 15 c.c. of medium. These were slanted and inoculated as above described and incubated at 22° C. Five similarly prepared cultures on each medium were included in every series. Periodically, usually twice a week, they were removed and examined in a moderately lighted room.

(1) *Fomes applanatus* (Pers.) Wallr.

Fomes applanatus is one of the most conspicuous and widely distributed of the pored fungi which attack our forest trees. White's (55) admirable summary of former records of its distribution obviates the necessity of a further review. From personal observation he reported the occurrence of its sporophores on living trunks of seventeen genera of dicotyledonous trees. His list of hosts included also Tsuga, Pinus and Picea among the conifers.

Material. Cultures here studied were started from sporophores obtained from dead *Abies balsamea* and living *Acer saccharum* received from Algonquin Park, Ont., and from wood and sporophores from *Ulmus americana*, *Tsuga canadensis* and *Fagus americana* collected in the vicinity of Toronto. Cultures started in May, 1920, have been continued by subculturing through fourteen series and have proved constantly characteristic and similar to those subsequently added from other sources.

Agars compared: Potato-dextrose, potato, corn, malt, Czapek's synthetic, Czapek's and Currie's synthetic (modified).

Habit of growth (Figs. 1 and 6): *Potato-dextrose.* The advancing hyphae penetrated the agar and spread rather rapidly over the surface. At first the advancing zone was narrow, appressed, and

sodden, but in a few days while still appressed it became somewhat silky in appearance, and in ten days the superficial growth was sufficiently rapid to keep pace with the surface layer so that a soft, downy-woolly texture was imparted to the zone of advance. The mat was throughout very tightly woven and except in the soft, earlier growth at the edge was closely appressed, rather thin and skin-like, with a felty or somewhat farinaceous surface. In cultures two weeks old the very close weaving of the surface layers caused the mat to roll at the edges (Fig. 1) and this rolling continued, breaking the penetrated layer of agar and exposing at the sides new surfaces, which were soon overrun by a soft felty growth. The surface of the mat was laden with a secretion of moisture which accumulated in large drops. In old cultures the surface lost its felty appearance and became dry and hard. Mats on *potato* were similar, but much thinner, and they remained flat. On *malt* the growth was quite as good as on potato-dextrose, but the mat was less closely woven. As a result it remained softer and did not roll. Growth on *Czapek's* and *Currie's synthetic agars* (*modified*) proved practically identical. Thicker but less extended mats than those on potato-dextrose were produced (Fig. 6). No appressed advancing zone was ever noted since the upper growth was rapid and as the culture developed formed a thick, soft band at the limits of advance. The mats did not become hard and skin-like but continued to form beautiful, very closely woven and dry felts. On *corn* growth was thin, filmy, and closely appressed, remained poor, and presented no distinctive characteristics. Development on *Czapek's synthetic agar* was also poor and did not advance far over the surface. It did not remain filmy as on corn, but formed hard, thin, irregular patches in the vicinity of the inoculum.

Color. On several media the coloration was rich and varied, appearing in zones or irregular patches, which deepened in tint as the cultures aged. On *potato-dextrose* the range in young cultures included vinaceous fawn and avellaneous. In the tube shown in Fig. 1 the central circle was of ecru olive, with zones of very pale ochraceous buff, vinaceous fawn, light cinnamon drab, cream color and white. Later development added shades of pinkish and tileul buff and darker tints of citrine drab and deep greyish olive. Coloration on *malt* presented a similar range. The mats on *potato* were more faintly colored, remained white during the first week's growth and later became tinted with the more delicate colors of the above range. Less variegated mats were produced on *Czapek's* and *Currie's synthetic* (*modified*) *agars* as is shown in Fig. 6. In this there was but a shadowing of cream buff toward the centre. Later development, however,

produced areas, usually in zoned formation, of tileul buff, vinaceous fawn, and deep olive buff. The color advanced along a definite line, leaving the upper white area abruptly marked off. *Corn* cultures remained white to colorless, and in a month's growth nothing deeper than cream color appeared on *Czapek's synthetic agar* mats. In very old cultures, however, deeper tints of the above range developed.

Rate of growth. Spread was rapid on *potato-dextrose*, *potato*, and *malt* agars. In eleven days slants of the first two media were almost covered (Fig. 1). On the synthetic media containing peptone advance was slow. At the end of a month the surfaces were little more than half covered, but the mats were thick and growth proceeded until the slants were eventually overgrown. Development on *corn* and *Czapek's synthetic agars* was extremely slow and poor and the media dried before the mycelium had advanced far.

Microscopic features. The advancing hyphae were delicate, hyaline, branched, septated threads with inconspicuous clamp connections (Fig. 58 *a* and *b*). They were mostly about 4 μ in width but hyphae 10 μ in diameter were found. Mycelium of similar type with threads usually 2 μ in width formed the early submerged layer. On these, deposits in the form of encrusting balls (Fig. 56) were abundant. In cultures a few days old the delicate advancing threads of the aerial mat became replaced by fine, hyaline hyphae, 1-4 μ in width, with comparatively thick walls and narrow lumina, the former being one-fifth to one-third of the total hyphal width. These were more or less branched, very sparingly septated and without clamp connections (Fig. 57). A few showed pigmentation but most of the coloration was found due to deposits lying between the threads and serving to cement them into a compact layer.

In older cultures the submerged hyphae penetrating the agar at right angles to the surface were found to terminate in large, irregularly swollen cells (Fig. 55), which in mass formed a compact layer at varying depths in the substratum. With age some were found devoid of contents, but many remained filled with fine protoplasmic contents, in which large, refractive granules were conspicuous. The thick-walled, fibre-like hyphae of the aerial growth showed dense anastomosis and interweaving in certain areas, which resulted in the formation of a very closely compacted mat with honey-combed appearance when viewed under the low power of the microscope. Toward or at the end of certain hyphae profuse and fine branching frequently resulted in the production of the witches' broom-like masses figured by Brefeld (7) but none were noted as represented by him upon the type of hypha bearing clamp connections.

The different media provided few hyphal variations. Development of the fibre-like threads was somewhat delayed on the synthetic media employed. On *Czapek's synthetic medium* clamp connections were much more numerous and conspicuous than on other media; and on *Czapek's synthetic medium (modified)*, owing to the less dense interweaving of the hyphae, terminal branch clusters were more easily observed.

(2) *Fomes fomentarius* (L.) Gill.

Fomes fomentarius is found widely distributed in temperate Europe and America as a destroyer of deciduous trees. It occurs on dead and living wood particularly of the beech, birch, maple, and poplar; and causes the decay of both heartwood and sapwood. Von Schrenk and Spaulding (47) and Hedgcock (28) refer briefly to its destructive activity and Rankin (40) includes it among beech and birch diseases, but no intensive study of the extent or method of its attack has been made.

Material. Cultures studied were made from sporophores received in May, 1920, from Algonquin Park, Ont., and from three collections made in the vicinity of Toronto during the summer of 1921. The former were taken from living *Betula lutea* and dead *Acer saccharum*, and the latter from dead *Betula lutea* found in different localities. The several cultures have proved to be easily identified by characteristics noted in the first mats, and the original 1920 mycelium, perpetuated by subculturing maintains its distinct peculiarities.

Agars compared: Potato-dextrose, potato, corn, malt, Czapek's synthetic, Czapek's and Currie's synthetic (modified).

Habit of growth: Potato-dextrose (Fig. 16). The advancing hyphae formed a colorless appressed zone behind which the growth thickened to form a close felty to farinaceous mat. After a week's growth there was a considerable felt with soft, smooth surface. On mats of ten to fourteen days or older deposits of moisture were noted in the vicinity of the inocula. As the cultures aged growth tended to become hard and crust-like, with a closely woven, smooth surface and tough, leathery texture. On *potato*, development was very similar to the above, but much thinner, and the moisture was exuded and disappeared earlier than on potato-dextrose. The surface also had a rougher, somewhat warted appearance, and the texture was soft and the mat easily torn. Final growth on *malt* resembled that on potato-dextrose very closely, but the looser interweaving of the hyphae produced a softer mat, less leathery in texture. *Czapek's* and *Currie's synthetic media (modified)* produced practically identical mats. On

each in five days the inoculum was closely felted, and surrounded by a narrow felty halo, which passed into a narrow, even, appressed advancing zone. As growth proceeded circular ridges developed and a farinaceous-felty spread. In old cultures sodden areas appeared in the thicker portions near the inocula. Development was extremely poor on *corn* and *Czapek's synthetic agar*. On the former the thin mat remained scarcely visible after ten days' growth and never formed more than a thin matted film; on the latter the mat remained scarcely visible throughout, but over the inoculum the growth thickened to form a soft downy ball.

Color. The color range included delicate pinkish tints and browns. On *potato-dextrose* in a developing mat there was always a soft white zone toward the limits of growth, and behind this pinkish buff or light ochraceous salmon deepened into browns of varying intensity including ochraceous tawny, cinnamon, sayal or Prout's brown. The moisture was chestnut in color. On *potato*, the pigmentation deepened through pinkish buff to light pinkish cinnamon, but did not intensify further. Coloration on *malt* was similar to that on potato-dextrose. Pigmentation on *Czapek's and Currie's* synthetic media (modified) was delicate. In two weeks the color had deepened through light pinkish cinnamon to cinnamon buff, but except for dark sodden lines and chestnut colored moisture drops no further darkening occurred. Mats on *corn* were white to colorless. On *Czapek's synthetic agar* the mycelium remained untinted, but the agar browned in the vicinity of the inoculum.

Rate of growth. The mats developed with moderate rapidity on *potato-dextrose*, *potato*, and *corn*, covering the agar slants of all three media in about three weeks. Growth on the last-named agar was, however, extremely poor. On the *synthetic* media advance was less rapid. At the end of four weeks the upper edge of the mats was still 1.5-2 cm. from the top of the slants, but growth continued and the surfaces were eventually covered.

Microscopic features. The first formed hyphae were delicate, hyaline threads, 2-7 μ wide, which showed abundant branching and septation, were furnished with clamp connections and contained finely granular contents (Fig. 63). These hyphae, modified on some agars, formed the bulk of the submerged mycelium and were present in more or less profusion in the surface mats, forming the advancing zones in all cultures. In most cases there were differentiated later long, fibre-like hyphae, with thick, colorless to greenish walls and colorless to dark brown contents. These were sparingly branched and septated, 1.5 to 5 μ wide and possessed no clamp connections

(Fig. 66). A short distance below the surface on potato-dextrose agar there was found a closely compacted layer, visible to the unaided eye, and found on examination to be composed of hyphae of the first type with a profusion of short branches (Fig. 64). The threads developed, on the whole, at right angles to the surface. They possessed colorless walls, slightly thicker than those of the advancing zone, and many irregular expansions filled in young mats with fine granular contents. Beneath this compact layer hyphae of the first type penetrated the agar in all directions; and in the superficial submerged growth above, similar threads were found intermingled with colorless, hyaline, fibre-like hyphae of the second type. The aerial growth of well-developed mats was formed almost exclusively of hyphae of the second type, which showed all gradations from colorless to deeply pigmented threads very tightly interwoven to form a tough felt. *Lack of dextrose* caused the production of a softer, thinner mat, which was formed of elements similar to those described above, but with fewer coarse threads. The fibre-like hyphae remained for the most part unpigmented and possessed more delicate walls. No densely compacted submerged layer developed. *Malt* produced a mat closely resembling that on potato-dextrose. The aerial growth was somewhat thinner and softer owing to the larger proportion of fine much-branched hyphae in the older portions; but coarse, deeply pigmented threads were also abundant. The compact, submerged layer was well developed. On *corn* the thin mat contained only hyphae of the first type scantily developed. On the two synthetic media containing *peptone* there was a rich development of delicate, hyaline threads bearing clamp connections. These were closely interwoven and cemented into a compact mat. In the aerial growth a few fibre-like hyphae formed, but these were not responsible for the entire coloration, since some of the thin walled type were filled with brownish contents. The hyphae of the submerged growth were well developed and vigorous and the contents of crushed threads exuded in bright refractive drops. *Czapek's synthetic agar* presented decided variations. Few fibre-like hyphae were produced, but the threads bearing clamp connections were decidedly thick walled and gnarled in appearance, with many irregular sac-like expansions (Fig. 65). They were 3-4 μ in width, much branched and septated, and possessed firm-walled, conspicuous clamp connections.

(3) *Fomes igniarius* (L.) Gill.

Fomes igniarius has a world-wide distribution as a wound parasite of deciduous trees. Its activity was first noted by Hartig (25) in his

study of oak diseases; and little beyond notes on its extensive distribution has been added by later investigators to his careful anatomical researches. References to its occurrence and observations on its action are made by von Schrenk and Spaulding (47), Atkinson (1), Hedgcock (27), Meinecke (34), Hesler and Whetzel (29), and Rankin (40).

Material. Cultures here studied have been obtained from several sporophores taken from living trees of each of the following hosts: *Fagus americana*, *Ulmus americana*, *Betula lutea*, *Betula alba*, *Acer saccharum*, *Ostrya virginiana*, *Populus grandidentata*, and *Populus tremuloides*. The uniformity of type of growth from different sources is illustrated in Figs. 36-40 in which slight differences in rate of growth and surface texture fail to mask the fundamental characteristics, except in the case of cultures obtained from Populus, which are described below.

Agars compared: Potato-dextrose, potato, malt, Czapek's synthetic, and Czapek's synthetic (modified).

Habit of growth. A week's growth on *potato-dextrose* agar produced a thick soft ball over the inoculum and a spreading area with downy-woolly surface. The mat, thickening markedly, soon tended to fill the base of the tube; and presented, especially in the older portions, a rough irregularly tufted surface (Fig. 11). Toward the limits of growth there appeared a very close, compact, but soft and woolly band, beyond which a light downy zone continued the advance of the mycelium. Amber yellow moisture drops formed here and there over the surface. *Omission of dextrose* caused a limitation of growth, the mat produced being noticeably thinner, but exactly similar in type. *Malt* also caused little variation. The spread of the mycelium was somewhat more rapid, and the mat rather thinner and softer, but the fundamental characteristics remained the same. On *Czapek's synthetic agar (modified)* advance of the mycelium was less rapid than on potato-dextrose, the surface somewhat smoother and studded at the end of three weeks with dark, russet brown moisture drops. On *Czapek's synthetic* medium development was less satisfactory, the thin, downy-filmy mat forming a rough woolly circle in the older growth but remaining light and loose over the remainder of the slant.

Color. The color ranged through a rich and varied series of yellowish and reddish browns, including: cartridge, cream and cinnamon buff, chamois, honey yellow, Isabella color, clay color, tawny olive, and cinnamon brown. Preceding the colored area in young cultures a white to cream buff zone continued the advance. On the four media yielding similar growth the coloration presented inter-

grading tints from the foregoing range. *Czapek's synthetic agar* produced an even colored mat, which after one week's growth was pure chamois but later deepened to an even honey yellow or yellow ocher. The agar of these cultures became discolored, intensifying through olive yellow to deep reddish brown; but the medium was never stained evenly throughout the entire depth, deep reddish brown in the superficial layer passing somewhat abruptly to yellowish green in the agar below.

Rate of growth. Advance of the mycelium was rather slow, but the thickness of the mats indicated the suitability of the media employed. *Potato-dextrose* and *potato* agar slants were covered in about three weeks, those of *malt* some days earlier, while on *Czapek's synthetic agar* (*modified*) growth was about a week behind the latter. Mats on *Czapek's synthetic agar* were neither thick nor rapid in spreading, but the thin filmy growth covered the agar surfaces by the end of a month.

Odor. The odor of wintergreen was noted in all cultures on *Czapek's synthetic agar*, and on Czapek's agar (modified) a rather disagreeable odor resembling that of sodium hydroxide was found characteristic.

Microscopic features. The first formed hyphae were delicate, hyaline threads, 2-4 μ in width, loosely branched and tangled, showing frequent septation, but lacking clamp connections (Fig. 90). These formed the greater part of the submerged mycelium and the advancing zone of the aerial mat. In a few days coarse, heavily walled, fibre-like hyphae were differentiated in the surface growth. These were somewhat sparingly branched and showed very infrequent septation (Fig. 93). The smooth, yellowish green walls were one-fifth to one-third of the total hyphal diameter in thickness, and the homogeneous contents yellowish to dark brown in color. The hyphae were 2-4 μ in width and very uniform in outline.

Sections through a mat of four weeks' growth (Fig. 89) showed a superficial layer of fibre-like threads. Nearer the agar the tangle of hyphae consisted of less deeply colored fibre-like threads intertwined with those of the first formed type. In the superficial agar layer, which was more or less stained, hyphal pigmentation gradually decreased. The much-branched, septated threads were often thicker-walled than those of the earlier growth, and densely tangled (Fig. 91). They usually formed at varying depths one or more thick, closely compacted layers of swollen cells (Fig. 92) firmly packed into a solid mass. With age these layers, becoming deeply stained, formed conspicuous almost black lines, beneath which delicate, hyaline threads

continued to penetrate the substratum. In some cultures the swollen cells, produced more sparingly, remained less prominent, but they appeared in greater or less abundance in all cultures. *Potato-dextrose, potato, malt,* and *Czapek's synthetic agar (modified)* produced the same type of growth, although the firmly woven layer just below the agar surface on the last-named medium consisted rather of gnarled, irregularly branched and closely matted threads with occasional spherical expansions. On *Czapek's synthetic agar* the fibre-like development was scanty and the browned, superficial agar layer contained a loose tangle of irregular hyphae with scattered but frequent expansions. The contents were yellowish, or dark reddish brown, single cells along the hyphae showing up abruptly due to their dense and darkly colored contents.

Variations. Cultures from various sources showed variations in texture, color intensity and rate of growth, which appeared in part individually constant but, except in the case of cultures from poplar, slight and unconnected with the host from which they were procured. The four strains from silver maple presented in one series the full range of texture variation. Four from white birch, on the other hand, were among the most rapid in development and produced rather smooth mats of delicate coloration; but the somewhat uniform variation fell within the range exhibited in other cultures. The necessity of considering peculiarities in relation to one another rather than stressing single characteristics is shown by a consideration of the extremes of variation in rate of growth in a series of cultures carried in duplicate from twenty-two sources, and observed after nine days' growth. In the cultures showing least development the mats had spread a distance of 2.3 and 2.8 cm. along the agar slants; at the opposite extreme were cultures showing 6 and 6.1 cm. advance. In texture they ranged from very roughly tufted to moderately smooth and felty-woolly, and in color presented either the deeper or lighter tints of the series above detailed or blended shades from both ends of the range.

(a) *Cultures obtained from poplar.* It has been stated previously that, with one exception, each of the fungi treated, although obtained from as wide a range of sources as possible, has yielded an easily recognized type of growth, which has been maintained through successive subcultures. The departure from such behaviour is presented by the growth of *Fomes igniarius* obtained from poplar. Cultures have been started from sporophores taken from two trees of *Populus tremuloides* and from one of *P. grandidentata.* The fruit bodies were typical of the species, but cultures from them presented

very interesting peculiarities in the course of their development. Long and Harsch (31) found such a difference in the growth of cultures obtained from sporophores taken from *Alnus* sp. and *Populus tremuloides* that in spite of the similarity of the fruit bodies they state that, "The cultural characters instantly showed the two were not the same species." In their preliminary report, however, the details are not presented, so that a comparison with their results is not yet possible. Initial growth obtained in my own cultures would certainly suggest the same conclusion, if species were to be founded on such incomplete cultural data without further consideration. Observations on several series of subcultures, however, throw doubt on the wisdom of such an instantaneous diagnosis, suggesting that the variations here noted, though marked, are scarcely of specific value.

The first cultures of the Populus strain obtained were started from a sporophore received in May, 1920, and were carried along with a series of the main species from twenty-one fruit bodies representing mycelium from seven different hosts. They alone showed noticeable variation from the type; but, although carried in stock series for some months, were not subjected to a critical study. Cultures from two sporophores taken from *Populus tremuloides* were received in September, 1921, and at once suggested the earlier growth. Both of these have been carried until the present and, while not identical, are easily recognized as the same strain.

Agars compared: Potato-dextrose, potato, malt, Czapek's synthetic agar, and Czapek's synthetic agar (modified).

Habit of growth. During the first week's growth development was so slow that scarcely any difference was noted in cultures on the several media. There resulted a thin, somewhat felty growth over the inoculum surrounded by a narrow appressed halo. Two weeks' development on *potato-dextrose* (Fig. 40) produced a thin, closely-woven circle about 1.5 cm. in diameter with a smooth farinaceous-velvety surface and narrow appressed advancing zone, and showed the agar stained for a considerable distance in the vicinity of the developing mat. The growth spread about 1 cm. a week during the first month's development, retaining its thin, compact structure and smooth surface. Growth on *potato* agar was similar but much thinner. Initial growth on *Czapek's synthetic agar (modified)* also closely resembled that on potato-dextrose, but in old cultures the mats differed considerably, becoming thickened irregularly with knobs and ridges although they retained a soft, velvety surface texture. Nothing but a thin scarcely visible film appeared on *Czapek's synthetic agar.* On *malt* there was produced a mat similar to that obtained on potato-

dextrose; but in this case growth was somewhat, although not markedly, more profuse and rapid. Before the end of a month, however, there were occasionally obtained mats resembling those previously described as characteristic of *Fomes igniarius* from other sources. These are discussed at greater length below.

Color. The color variation agreed for the most part with that displayed by mats from other sources, and included on *potato-dextrose*, *potato*, *malt* and *Czapek's synthetic* agars shades from the range of chamois, through Isabella and clay color, to cinnamon brown. On *Czapek's synthetic agar (modified)* deeper tints appeared. Beginning in the first week with buckthorn brown, chamois, and white, the coloration had intensified by the end of a month to include raw umber, antique brown, yellow ocher, and Mars yellow, with a rim of white. The agar, except in the case of potato, stained deeply; and even in the case of potato, slight discoloration was noted.

Rate of growth. As indicated above the advance of the mycelium was extremely slow. On the five agars used measurements at the end of four weeks' growth gave the following as the extent of the mats along the agar surfaces: *potato-dextrose* 3 cm., *potato* 2.5 cm., *malt* 5.3 cm., *Czapek's synthetic* 2 cm., and *Czapek's synthetic (modified)* 2.5 cm.

Variations in growth. Periodical observations were not made on cultures older than a month; but in stock series run on potato-dextrose or malt it was noted that, while the closely-compacted and somewhat crust-like mat never covered the agar surfaces, there often developed a softer, velvety to downy growth, which eventually advanced to the limits of the agar slants. When present it assumed tints passing from cinnamon brown through chamois to white.

As indicated above, reversion to typical *Fomes igniarius* growth was occasionally obtained on *malt*. Such mats were also produced, though rarely, on *potato-dextrose*; but in this case the change of type was never initiated during the first month's growth. On the latter medium the first instance of such development was noted in the seventh subculture after the running of six series on potato-dextrose or malt, in which the cultures, from which inocula were taken, contained only the slow growing, crust-like type. It appeared in one of two cultures similar in source and age, which, having been examined and found typical for poplar cultures during the first month's growth, were set aside and not subjected to further study for about two months. One of the tubes was then found to contain a mat, somewhat dried, but quite typical of *Fomes igniarius* from other sources. Subculturing from this tube, six tubes inoculated using as inocula

either superficial growth alone or submerged mycelium freed as well as possible from the aerial mat, produced without delay growth presenting peculiarities similar to those above detailed as characteristic of *Fomes igniarius*. The mats were thick and soft, with tufted woolly surface and the typical coloration ranging from Isabella color to cream buff. On *malt*, though not the usual type, such development was by no means rare, and, as before mentioned, occasionally occurred during the first month's growth. No explanation of the appearance of such mats presents itself; they developed quite irregularly, sister tubes often producing the two types of growth. They were not used to perpetuate the strain, subcultures for stock series being started in every case from the compact, crust-like type; and the developing mats produced always at first the thin, slow-growing felts. Hyphae initiating the change in texture appeared as far as noted in the advancing zone and gradually spread until the whole surface presented the thick mat previously found typical of the species.

Microscopic features. In the hard, crust-like mats the advancing hyphae were moderately strong-walled, regular in outline, 2-3 μ wide, branched, septated, and, especially in old cultures, irregularly pigmented, dark yellowish or reddish brown segments appearing in the otherwise colorless hyphae. In the older growth the threads were similar in type but much more profusely branched and matted, showed more general and intense pigmentation, and measured 5-6 μ in diameter. The submerged mycelium was also similar, but the hyphae were mostly 2 μ in width and were not profusely branched. The hyphal contents were yellowish or, toward the surface, reddish brown and the agar showed discoloration. Cultures on all media produced hyphae very similar in type. On *Czapek's synthetic agar* the hyphae measured 1-5 μ in diameter. Individually they were vigorous in appearance, but were not developed in profusion. The soft surface growth on *Czapek's synthetic agar (modified)* was found due simply to the looser interweaving and less dense branching of hyphae of the ordinary form.

The light downy-velvety growth found spreading in old cultures from the limits of the crust-like region was composed of fibre-like threads, very sparingly branched and septated, intermingled with the more delicate, septated hyphae, which are characteristic of the species. Microscopically this area agreed with ordinary *Fomes igniarius* growth, but the texture in mass was much lighter and looser. The pale downy upper growth contained chiefly colorless hyphae 1-4 μ in width, in which large, irregular, nodular swellings with dense, dark

brown contents were abundant and conspicuous. A few pigmented fibres were present, and many of the hyphae had dried and collapsed.

Cultures from poplar were thus found to differ from those from other sources in two important respects. In the former, production of fibre-like hyphae was uncertain and usually long delayed, while the septated mycelium was densely matted and deeply though irregularly pigmented; in the latter, fibre-like threads were produced readily and in abundance, while the septated hyphae remained for the most part colorless. In the latter also the production of compact, submerged layers of enlarged cells was characteristic. The rather rare mats from poplar, which agreed macroscopically with those from other sources, presented also the typical microscopic peculiarities.

(4) *Fomes pinicola* (Sw.) Cooke.

Fomes pinicola is a common enemy of coniferous wood and is found, though less frequently, on several species among deciduous trees. No certain demonstration of its parasitic activity has been furnished but the occasional occurrence of its sporophores on living trees points to the possibility of its power of attacking living tissues. Atkinson (1) states that, "The fruit bodies are sometimes found on the trunks of living trees, but much more frequently they do not appear until the tree is dead." Von Schrenk and Spaulding (47) consider it essentially a wound parasite, and found that living trees bearing its sporophores were always weakened or sickly, but whether from the action of this or another organism they did not determine. Hedgcock (28) states that it attacks living trees to some extent, entering through wounds and attacking either heartwood or sapwood, both of which it destroys readily. Meinecke (34) finds it the active agent in production of red rot in the heartwood of most conifers in California, where it occasionally damages living trees and is very destructive to fallen timber. Rankin (40) says, "It is thought that the fungus usually attacks living trees which are badly wounded or in generally poor health." Faull (21) finds it saprophytic in northern Ontario, where it is abundant on dead *Picea mariana* and the cause of heart rot in the same host. In any tree its uncontrolled development results in the complete destruction of woody tissues.

Material. Cultures were obtained from sporophores and wood from dead *Populus grandidentata, Betula alba* and *Abies balsamea,* and from sporophores from dead *Pinus strobus, Picea mariana, Larix americana, Tsuga canadensis,* and *Prunus serotina.* Cultures were also started from sporophores from living *Abies balsamea.*

Agars compared: Potato-dextrose, potato, corn, malt, Czapek's synthetic, Czapek's and Currie's synthetic (modified).

Habit of growth. The mats on all agars were characterized by great delicacy of texture and coloration. They were soft and easily torn. Developing mats on potato-dextrose were, owing perhaps to the greater abundance of fibre-like threads, slightly tougher than those on other media, but were themselves delicate. They all separated readily from the submerged mycelium, leaving a smooth agar surface, and showed no discoloration on bruising. On *potato-dextrose* (Fig. 3) the advancing zone was loose downy to downy-silky, no appressed band appearing since the aerial growth was sufficiently rapid to keep pace with the surface mycelium. In four days there was a thin, dry, downy mat, which gradually thickened to form a close downy-felty area in the older growth but remained light downy toward the top of the slants. In two weeks the mats presented soft, even, felty-woolly to matted surfaces, which later became more matted and often somewhat loosely honey-combed. *Potato* and *malt* produced mats practically identical with those on potato-dextrose. On *Czapek's* and *Currie's* synthetic agars (modified) (Fig. 8) growth was similar but more compact and even than on potato-dextrose. The initial growth was soft, thick, and silky. This became appressed with felty-woolly surface and a downy silky advancing zone, which, extended in a straight line across the slant, continued the spread. Later growth made the mats very similar to those on potato-dextrose except that cultures after three weeks' growth had large deposits of moisture over their surfaces. On *corn* the mats resembled those on potato-dextrose, but were much thinner and became laden with tiny, clear, colorless drops of moisture. Beginning with a dry, appressed, somewhat farinaceous spread, growth on *Czapek's synthetic agar* advanced with a very light downy zone and developed to form a somewhat floccose mat, which later thickened irregularly, leaving filmy areas here and there. The growth was dry throughout.

Color. The color range included only tints of extreme delicacy. The deepest coloration was obtained in cultures ten days to two weeks old; they then gradually faded. On *potato-dextrose* the mats were white shadowed with pale ochraceous buff to pale ochraceous salmon and then became dull. *Potato* and *malt* produced pigmentation of similar intensity. On the media containing *peptone* the deepest coloration developed. On these the mats, at first shadowed with pale ochraceous buff, intensified to a light ochraceous salmon and then gradually faded through pale ochraceous salmon to pinkish buff.

Mats on *corn* and *Czapek's synthetic* agars remained pure white throughout.

Rate of growth. Advance was rapid, slants of *potato-dextrose, potato, malt* and *corn* being almost covered in eleven days' growth. In seventeen days the surfaces of all media were covered, but, as before noted, growth on *corn* and *Czapek's synthetic* agars was never luxuriant.

Microscopic features. Initial growth consisted of a very delicate type of irregularly branched, colorless hyphae with frequent septa and numerous clamp connections. These were very fine, 1-2 μ or occasionally 4 μ wide, had very delicate walls and rather coarse, granular contents. In the submerged mycelium the same type of hypha appeared; but they were, on the whole, broader and more vigorous in appearance: 2-6 μ, with the majority 4 μ wide. On potato-dextrose before the end of the first week there developed thick-walled, fibre-like threads with narrow lumina. These were colorless, very sparingly branched, uniform in width, unseptated, and with hyaline walls of uniform thickness. They also were fine, usually 2 μ but ranging from 1-4 μ in width. Both types were formed on all media except *corn*, on which the entire mat consisted of delicate, thin-walled threads with septa and clamp connections; but on *potato-dextrose* the fibre-like hyphae developed earlier and were formed in greater profusion than on other media. Even in old cultures on *malt* the bulk of the aerial growth consisted of very fine threads of the first type. These delicate hyphae were rendered more conspicuous on *Czapek's synthetic* agar owing to the development of somewhat thicker walls than in other mats, and the aerial growth on this medium was found powdered with a mass of fine crystals.

Variations. The first cultures obtained were started from sporophores from living *Abies balsamea* in May, 1920, and produced the typical mycelium described above. Since February, 1921, when the bulk of the material from which cultures have been made was collected, nine series have been observed. Individual differences have been noted in the several sets, which made it possible to pick out cultures from particular sources, but the range of variation was so slight that there was no difficulty in recognizing any of the cultures as those of *Fomes pinicola.* No indication of host influence in the production of the slight, constant variations was noted. Cultures from sporophores from balsam and spruce proved indistinguishable, while those from two spruce trees were sufficiently distinct to be selected as derived from different sources. Each series contained two or more tubes from each source, and the corresponding tubes were separated in

the case mentioned simply by a slight but distinct difference in color intensity. On the other hand, differences in rate of color change rendered cultures from white pine, which were at first indistinguishable from those from one spruce tree, at a later date practically identical with the more deeply pigmented mats from the second spruce. The whole range of color variation was included in delicate shadowing of pale ivory yellow or very pale ochraceous buff through pale ochraceous buff to pale ochraceous salmon. Slight differences in rate of spread and in thickness of mat were also evident, but were insufficient to cause confusion. These minor variations diminished with age and in old cultures practically identical mats were obtained from all sources.

In the second subcultures from one of the sporophores obtained from hemlock daedaloid surfaces about 1 x 1.5 cm. in extent developed at the upper edge of the agar slants. These were formed in both tubes of the stock series and were duplicated in the following subcultures. When first examined they gave the impression of rudimentary fruiting surfaces; but, if such were the case, the environmental conditions provided did not further the development and they gradually dried. One of them sectioned showed an underlying aerial layer composed of closely intertwined fibre-like hyphae, covered by a less densely interwoven layer, which passed into the superficial pored covering about 0.5 mm. in thickness, in which the anastomosing walls were of similar, tightly interwoven strong-walled threads. No indication of a developing hymenium was noted, however; nor, indeed, were threads suggesting fertile hyphae found in the interwoven hyphae. Cultures of *Fomes pinicola* were started from nineteen sources in all, but no other strain gave any suggestion of even the rudiments of fructification.

(5) *Fomes roseus* (Alb. & Schw.) Cooke.

Fomes roseus is a fungus common in both Europe and America. Hedgcock (28) reports its practically universal distribution throughout the United States, where it appears chiefly as a destroyer of coniferous wood; but he notes its occurrence also on the trunks of peach, birch, and red maple. Von Schrenk (44) records it as a cause of pocket heart rot in juniper. Hedgcock confirms his findings, and states it to be an active agent in the decay of living spruce and juniper. He finds it also as the cause of a rot of cedar, which may be indistinguishable from that caused by *Polyporus schweinitzii*. Its sporophores are found commonly on dead areas of living trees, or on fallen logs in which both heartwood and sapwood may be attacked. Faull (21) reports it from northern Ontario as 'fairly common' on dead *Pinus resinosa*, and 'occasional' on dead *Picea mariana*, but finds no

evidence of its activity as a cause of heart rot. Snell (49) has included it among the five destroyers of structural timber, which he has distinguished by cultural characteristics. There are in the Herbarium of the University of Toronto specimens of Douglas fir showing heartwood rot due to this organism, which were collected by J. S. Boyce in the State of Washington.

Material. The cultures here studied were started from the tissue of sporophores collected from dead *Picea mariana* in September, 1921. Grown under standardized conditions they have proved through successive subcultures remarkably uniform in both microscopic and macroscopic characteristics. Snell (49) states that his tissue cultures have varied from transfer to transfer both in color and character of growth, but of the cultures here considered sets of from four to six tubes of the same age and upon the same medium have in many series been found so similar as to be altogether indistinguishable. Frequent subculturing also has produced no noticeable variation.

Agars compared: Potato-dextrose, potato, malt, Czapek's synthetic and Czapek's synthetic (modified).

Habit of growth. The early growth on *potato-dextrose* was a fairly close, light, flocculent-downy mat, which with age became closely woven and produced a generally even, woolly-felty surface (Fig. 10). Mats of similar type, but with surface more matted and dried, developed on *Czapek's synthetic agar (modified)*. During the first week growth on *malt* was very like that on potato-dextrose, but later the mats became much softer and more downy in appearance, due to a looser interweaving of the hyphae. On *potato* the first growth was thin, filmy and somewhat farinaceous, and as the mycelium spread a considerable area remained farinaceous-downy and sufficiently thin to leave the agar visible. The oldest growth near the inoculum formed a thin, hard circle, and toward the top of the slant the downy mycelium thickened to a light, soft mat. The first week's growth on *Czapek's synthetic agar* consisted of a thin furry film, and as this extended the newer growth formed a close, light downy area, while near the inoculum the mat thickened and hardened, retaining, however, a light surface growth.

Color. On *potato-dextrose*, during the first week's growth the mats remained white with the exception of an occasional excretion of tiny moisture drops over the inoculum. Gradually color appeared and deepened. In three weeks there was a rather general shadowing of light ochraceous salmon, which intensified later to light vinaceous cinnamon and reached at the end of a month an even, almost vinaceous buff with staining of avellaneous. The agar stained as growth pro-

ceeded, assuming at first a pinkish tint, but darkening finally to almost black. On *potato* most of the mat remained white throughout, but the older growth encircling the inoculum had at the end of a month produced a faint pinkish buff coloration. On *malt* the color proved similar to that on potato; but, owing to marked differences in texture, the cultures remained distinct. *Czapek's synthetic agar* gave the earliest and deepest coloration. Beginning in the first week with a rather general pinkish shadowing the color deepened gradually producing russet to cinnamon shades in the older and harder growth, and in the newer, light ochraceous salmon finally dulled to light pinkish cinnamon. The initial pinkish coloring of the first week's growth on *Czapek's synthetic agar (modified)* became gradually dull and dark, until at the end of three weeks the mat had assumed an almost even avellaneous tint, which dulled as the culture dried. The agar became black.

Rate of growth. The mycelium advanced rather rapidly and uniformly on the five media used, covering in each case about 4.5 cm. of the slant in the first week's growth. The area covered was slightly greater on the potato medium lacking dextrose. *Potato-dextrose, malt,* and *Czapek's synthetic agar (modified)* gave similar mats somewhat thicker than those on *potato* and *Czapek's synthetic* agars, on which the early growth was filmy. At the end of a month, growth on potato-dextrose was thickest and most closely woven; malt and the peptone medium showed only slightly less development; but Czapek's synthetic and potato agars gave noticeably thinner growth.

Microscopic features. Growth spread gradually from the inoculum and penetrated the agar. Initial growth, both aerial and submerged, consisted of one type of hypha: branched, septated, turgid, colorless threads, 1-3 μ in width, furnished abundantly with clamp connections. On *potato-dextrose,* as growth increased, the hyphae became massed into a compact mat just below the agar surface. The original type of hypha still formed for some time the predominating element, but differentiation gradually occurred. Hyphae similar in form but conspicuous owing to width (about 6 μ in diameter), irregularity of outline, and scarcity of contents were found traversing the mat. Others resembling in type and size the original hyphae became conspicuous owing to their dense, darkly pigmented, coarsely granular contents. These were most abundant in the aerial mycelium, but extended also into the submerged. In all types bearing clamp connections anastomosis of branches was not uncommon. Fibre-like threads were gradually developed in the aerial mycelium. They averaged 2-3 μ in diameter, were usually sparingly branched, had thick, refractive,

hyaline walls, few septa, no clamp connections, and no pigment. None were noted in cultures two weeks old, but towards the end of the third week they were well formed. Similar elements constituted the mats developed on *potato* agar, but fibre-like threads were infrequent even toward the end of the fourth week, and pigmentation was insufficient to render any of the hyphae conspicuous. The farinaceous appearance was due to deposits of crystals soluble in hydrochloric acid. On *malt* pigmentation was rather generally diffused through the aerial growth and was not deep; fibre-like threads were not abundantly developed and appeared late. *Czapek's synthetic agar* presented in the light aerial growth an abundance of fibre-like threads mingled with delicate, hyaline hyphae bearing clamp connections. Of the latter type heavily-walled threads were also noted with their rather homogeneous contents uniformly, but not deeply, pigmented. In the older growth the pigmented hyphae were dense, and dark, and usually showed the coarse granulation before noted; but the walls were somewhat thickened, giving them, where unbranched and without clamp connections, the appearance of fibres. On *Czapek's synthetic agar (modified)* the mycelial layer at the agar surface became densely compacted, formed of rather uniform much-branched threads with hyaline walls and numerous clamp connections. Mingled with these in greater or less abundance were the deeply pigmented hyphae. No fibre-like threads were noted. The light aerial growth and submerged layer consisted of delicate hyaline hyphae as found on other media.

(6) *Polyporus borealis* Fr.

Polyporus borealis ranges widely in both Europe and America as a parasite of conifers, but seems never to occur abundantly in any locality. In 1878 Hartig (25) discussed its activity in the production of a heartwood rot of *Picea excelsa*. Later Atkinson (1) recorded its occurrence and mode of attack as a wound parasite of *Tsuga canadensis* and *Picea rubra* in the state of New York. Faull (21) has reported it as a rather infrequent cause of heartwood rot in *Abies balsamea* in northern Ontario.

Material. Cultures described below were set up in August, 1921, from sporophores and decayed wood of *Picea mariana* collected in the Timagami Forest Reserve, Ontario, from dead wood of *Picea* received from Quebec, from sporophores from *Tsuga canadensis* found in the vicinity of Toronto; and at the same time transfers were made from cultures received from Mr. A. W. Macallum of Ottawa. The tubes, examined during some weeks, presented a uniform and unique growth

as detailed below, and subcultures have been easily recognized during the year they have been under observation.

Habit of growth. On *potato-dextrose* (Fig. 50) the mat spread with a colorless, appressed advancing zone. After a week's growth there was a soft, even felt over the inoculum, which passed into a light, flocculent zone. The mat thickened rapidly toward the bottom of the slant, tending to fill the base of the tube. This lower growth was soft and thick, but toward the top of the tube even after three weeks' growth the surface layer was thin with farinaceous surface. Irregular felty balls and ridges were often formed and from these moisture exuded. The thicker growth retained its soft, felty to felty-velvety surface and the thinner area its farinaceous appearance until the culture dried with age. *Omission of dextrose* caused a diminished growth and produced a mat of softer texture. On *malt*, development was more luxuriant and the mat softer than on potato-dextrose. In the first week there was produced a downy-felty covering over the inoculum with a downy-silky spread, which passed into a silky advancing zone. The type of growth, however, resembled on the whole, that on potato-dextrose. The bases of the tubes were plugged and in the upper part there formed a thin, felty-woolly mat, while the diminution in rate of spread left visible at the end of two weeks a colorless, appressed advancing zone. Over the irregularly thickened surface there was a great exudation of moisture, which dried by the end of the third week. At that date the appressed advancing zone was fully 1 cm. in width. Growth increased in thickness but spread slowly. The thickened, somewhat downy area was covered with irregular soft balls and tufts, while the upper part of the slant became covered with a close, smooth and farinaceous to felty growth. Some tubes produced fruiting surfaces as described below. *Czapek's synthetic agar* produced a mat, which advanced with a thin, spreading, appressed, colorless, silky zone and the rest of the mat continued farinaceous-flocculent to farinaceous-felty throughout the first month's development. Old cultures possessed a thin, dry, matted woolly growth. Growth on *Czapek's synthetic agar (modified)* resembled that on potato-dextrose but the mats were thinner and developed more slowly. The appressed, advancing zone was narrow and the mat closely woven. At the end of four weeks the bases of the tubes were almost filled with a soft growth with close shaggy-woolly surface; and in old cultures the mat was like a cotton plug filling the tube to the upper edge of the agar surface.

Color. On *Czapek's synthetic agar* the mats were pure white throughout; on other media delicate cream color tinting developed.

Rate of growth. Advance was slow, but owing to the rapid thickening on most media the amount of growth was considerable. The most rapid and best development was obtained on *malt.* On this the slants were covered in three weeks and the bases of the tubes were well filled. Slants of *Czapek's synthetic* agar were also covered in three weeks but the mats were thin. On all other media the agar surfaces were covered in four weeks.

Microscopic features. The hyphae which first penetrated the agar and spread over the surface were fine, firm, regular, much-branched, septated, and furnished abundantly with clamp connections. They were 2-4 μ wide; had uniform, firm, colorless, hyaline, refractive walls; contained dense, granular, protoplasmic contents; and produced numerous short side branches. In older mats on all media hyphae of this type, 2-6 μ wide, formed the submerged mycelium and a considerable part of the aerial growth. These always produced an abundance of chlamydospores, which developed in terminal or intercalary position (Fig. 78). The spores were spherical, subspherical, elliptical, pear-shaped or irregular; they had smooth, colorless, hyaline walls; their contents were dense and coarsely granular; and they were 6-10 x 10-20 μ in size. Although present in both submerged and surface growth they were more abundant in the former, especially in old cultures. In these, much of the growth was often formed of hyphae of the type described with somewhat thicker walls and devoid of contents. There were also developed sooner or later, and on some media quite abundantly, fibre-like threads, which were sparingly septated, more or less branched, and provided with very few clamp connections (Fig. 79). These were distinguished from empty hyphae of the first type by their proportionally narrower lumina and thicker walls, less frequent branching and septation and the very infrequent occurrence of clamp connections. They showed also greater irregularity of outline. They were produced in the older, felted areas. On *malt,* which permitted the most rapid growth, they were usually present in cultures one to two weeks old; on *potato-dextrose* they were usually at least a week later in developing; and on *potato* were infrequent after a month's growth. They were not found on the synthetic media used even at the end of a month, but in old cultures were obtained in limited numbers. Old aerial mats on *Czapek's synthetic medium (modified)* were formed almost entirely of empty hyphae of type one; on *Czapek's synthetic* agar similar hyphae were found, but were richly furnished with coarse, granular contents, and, intermingled with them, a few fibre-like threads.

Soft balls were developed over the surfaces of the mats, especially on potato-dextrose and malt. The interior of these was composed of closely interwoven hyphae of the types above described. The velvety or tufted surface was formed of parallel hyphae, which adhered in strands and developed at right angles to the surface. These were of the first type and were rich in protoplasmic contents. Some such surfaces on malt were found bearing irregularly distributed basidia with spores.

Sporophores. Fructifications, more or less normal in type, developed in several cultures from different sources. The first fruit body obtained was noted on October 15 in a potato-dextrose culture given me by Dr. J. H. Faull early in September, and started by him on August 23 from a sporophore taken from dead *Tsuga canadensis*. This was placed in a horizontal position with pored surface down in the hope of obtaining a spore cast; and two days later a visible cloud of spores was noted on the opposite side of the tube (Fig. 45). The fruit body measured 0.8 x 0.8 x 0.4 mm., and presented a surface of well-formed pores with somewhat angular mouths 0.5-1 mm. or less in diameter. The spore cast, as may be noted in Fig. 45, was thick. It was composed of typical, smooth, colorless, hyaline spores, subspherical to ovoid in outline, filled with coarse, granular contents, and 5-7 x 3-4 μ in size.

In all, ten sporophores were obtained in cultures between August, 1921, to September, 1922. Out of seventy-nine tube cultures fructifications developed in nine, and of six flask cultures one was productive. Of the tube cultures thirty-two were on potato-dextrose, twenty-three on malt, and eight on each of potato, Czapek's synthetic, and Czapek's synthetic (modified) agars. The first three media produced sporophores as follows: potato-dextrose five, malt three, and potato one. The tenth fruit body appeared on an upright potato-dextrose agar surface in an erlenmeyer flask culture. Of a series of six flasks containing potato-dextrose two were placed in diffuse light, two in darkness at room temperature, and the remaining two were incubated at 22° C. All were unproductive except one of those placed in the dark at varying room temperature. Incubation was, however, no bar to fructification since three of the tubes in which sporophores appeared belonged to series maintained at 22° C. All fruit bodies produced developed in comparative darkness, the tubes having been kept in thermostats or in closed cardboard boxes.

The sporophores varied in form from the regular pored type represented in Fig. 45 to irregular surfaces formed of anastomosing lamellae. All, however, produced a typical hymenial surface of

basidia bearing basidiospores and broad, tapering cystidia with distal ends heavily walled (Fig. 80). The viability of spores from a cast similar to that figured in Fig. 45 was tested in hanging drop and plate cultures. In the former the drops were of distilled water, 1% malt, or 1% dextrose and in each germination was readily obtained. Plates, using a 1½% malt with 1½% agar medium, were poured and kept at room temperature or incubated at 22° C. In the latter, germination was more rapid and in three days practically all the spores observed had produced primary mycelia as represented in Fig. 77 *a-d*. The spores after marked enlargement produced one or two germ tubes, which soon branched to form a septated, unclamped, primary mat. The first mycelium was in all cases noted submerged, and aerial growth was not obtained for some days. In nine days, however, good surface development was found in progress. No attempt was made to procure monosporous cultures. Tubes inoculated from the plates referred to produced the typical mats above described, composed of hyphae bearing clamp connections, numerous chlamydospores, and in old mats hyaline, fibre-like threads.

(7) *Polyporus schweinitzii* Fr.

Sporophores of *Polyporus schweinitzii* are found rather generally distributed throughout temperate Europe and America. They occur on or near the bases of trees and in the latter case are found to have developed from mycelium contained in the roots. Hartig (25) included its destructive action on pine wood in his studies of conifer rots, and in addition described in detail the structure and development of fruit bodies. Von Schrenk (45) found it very common in the north-eastern forests of America, where its fruit bodies were present in numbers throughout the summer on spruce and fir. He reported it as much more prevalent in America than in Europe, and as standing near the top of the list in point of destructiveness. It was found on White and Red Spruce, Balsam Fir, Arbor Vitae, and White Pine. Hedgcock (28) reported it as the most important root-rotting fungus found on conifers in Idaho, Montana, and Oregon, and as the cause of the loss of 5-30% of the Douglas Fir in those states.

Material. The first cultures obtained were started in July, 1921, from three sporophores collected in the Timagami Forest Reserve, Ontario. To these were added later spore and fruit body tissue cultures from a sporophore which developed near a stump in the Botanical Gardens, Toronto, and others from a fruit body obtained from living *Pinus strobus* in the Timagami Forest Reserve. Tubes containing mycelium were also received from Mr. A. W. Macallum

of Ottawa. Cultures from such varied sources have proved so similar as to leave no doubt as to the unique peculiarities of the organism in pure culture.

Spores. From a sporophore growing in the gardens adjacent to the Botanical Laboratories spores were collected throughout the period of spore fall: an interval of twenty-nine days during which traps were set daily. On September 22nd no spores were obtained, but the following day and until the sporophore was found broken off on October 18 they were collected in abundance. The fruit body was then brought into the Laboratory, where it continued to shed spores until the 21st.

Hanging drop cultures were started from several collections using the following media: distilled water, 1% dextrose, 1% sucrose, a weak solution of malt, decoctions of carrot, and of potato with and without dextrose. Plates of potato-dextrose agar were exposed to falling spores and others were poured after inoculation. The several series were carried in duplicate, and incubated at 22°, 27°, and 30° C. Besides inoculations with fresh spores cultures were attempted using material stored on glass strips on phials, and kept for some months under laboratory or outdoor conditions. In none of these, however, was germination obtained. The only spores which germinated were those sown on the surfaces of potato-dextrose agar slants. Cultures were started immediately after the spores were brought into the laboratory, and twelve days later round spreading patches of mycelium were noted on the agar surfaces. These merged and eventually produced the mat typical of the tissue cultures of *Polyporus schweinitzii.*

Media used. Growth in the original tissue cultures started early in July on potato-dextrose agar was so unsatisfactory that an attempt to obtain a more favorable substratum led to trial of the following media: Potato-dextrose −30, −20, −10, neutral, +10 and +20; beet; oatmeal; bean; corn; pea; celery; and carrot. Duplicate tubes of each were carried. Examination of these at the end of ten days showed characteristic variations in response to change of medium, but introduced no peculiarities sufficiently striking to make necessary or desirable the employment of any for diagnostic purposes.

(1) *Potato-dextrose* −30, −20, −10, neutral: no growth.

(2) *Beet,* and *oatmeal:* narrow halo, extending about 1 mm. from the inoculum.

(3) *Bean:* extremely thin, scarcely visible spread, clouded with farinaceous-woolly patches faintly cream in color.

(4) *Corn:* thin, irregular growth, composed of a farinaceous-woolly circle around the inoculum, and a filmy, scarcely visible spread with

thin, farinaceous-woolly patches; thickened areas colored chamois to cream buff.

(5) *Pea:* thin, downy-flocculent to loose woolly spread, tinted with patches of cartridge buff to straw yellow.

(6) *Celery:* thin, but distinct and even farinaceous-woolly spread of ivory yellow.

(7) *Potato-dextrose +10:* very good, though rather thin, close, even growth with farinaceous to farinaceous-woolly surface; older growth Naples yellow, and pale, thin, filmy advancing zone.

Potato-dextrose +20: excellent growth; distinct, fairly thick mat with tufted appearance over the inoculum, and farinaceous-woolly spread; upper growth rather thick, woolly, and tufted; coloration, Naples yellow shading to cream color.

Carrot: best growth of series; thick in comparison with the rest and close; thick circle near the inoculum with farinaceous-woolly surface and chamois to cream buff coloration, extending to a rough, woolly area pale greenish in color; mat thin and white toward the limits of growth.

Microscopic examination of mats from the last three agars, which were proving the most satisfactory of the series, revealed the typical mycelium to be described below.

The cultures above described were maintained at room temperature in almost complete darkness as were the originals from which they were started, and which had shown such poor development. In the later series the more luxuriant growth on potato-dextrose +10, which corresponded most closely with the first medium employed, is to be attributed to the lower temperature prevailing during the latter part of July when these cultures were under observation. During the earlier part of the month high temperatures were experienced, the thermometer standing between 30° and 36° C. for some days. As previously recorded, subsequent temperature tests on the growth of *Polyporus schweinitzii* has revealed the necessity of a lower temperature for rapid development. Since the potato-dextrose medium, although not the best of the series, was thus found to permit under favorable conditions a fairly active growth, and since it had been chosen for comparative work, its use was continued.

The series serves to show that the employment of numerous vegetable agars is of little moment in the production of marked diagnostic characteristics. Apart from variations in amount and rate of growth, and slight differences in color, no results were obtained. On all agars permitting development the type of growth and general coloration, a range of yellow tints, were very similar.

The extreme sensitivity of the mycelium of this fungus to the action of light was evidenced by the variation shown in series kept in a thermostat, but removed twice a week and exposed for examination in a moderately lighted room during a period of about half an hour, as compared with others which were allowed to develop in the thermostat for a prolonged period without removal. Since it is the only mycelium here discussed which showed noticeable change under such treatment, no more strict elimination of light was deemed essential during the period of examination, and the description here given for *Polyporus schweinitzii* deals with cultures similarly exposed. It is to be noted, however, that darkness, as mentioned in the discussion of the action of light, caused the development of the pale yellow, furry-filmy spread above described, with its thickened and more deeply colored patches, while the periodic exposure produced a somewhat zonated, velvety, and more vividly colored mat, which approached in type the development obtained under prolonged exposure.

Media compared: Potato-dextrose, potato, corn, malt, Czapek's synthetic, Czapek's and Currie's synthetic (modified).

Habit of growth. Early growth on *potato-dextrose* was thin, close and furry, with the spreading area filmy-farinaceous. During the second week's development the older growth formed a smooth, velvety circle around the inoculum. Exposure of young mats for examination caused a retardation and slight thickening of the growth producing close, felty circles. The newer zones at first downy-woolly became velvety with age, and the zonated appearance was gradually lost as the velvety surface became more widely spread and compacted. In the upper part of the tubes, soft fluffy balls developed and gradually became compact and velvety. The bases of the tubes were also filled with a similar growth. *Omission of dextrose* effected the development of a similar, but much thinner mat, while growth on malt differed only in its more rapid spread and profuse development. *Corn* produced a thin, colorless, almost invisible film which later became banded with downy clouds. Growth on *Czapek's synthetic agar* resembled that on corn, but with age velvety patches appeared near the inoculum. The mats on *peptone* media were exactly alike in type and closely resembled growth on potato-dextrose. They were, however, less rapid in development, presenting a felty-velvety surface with less brilliant coloration.

Color. On *potato-dextrose* the first greyish-white to colorless mycelium gradually gave place to a vividly colored mat of varied yellow tints darkened later by rich brownish shades. Ten days' growth produced a mat in which coloration decreased in intensity from older

to newer areas from sulphine to strontian yellow, through citron and barium yellow, to a white to colorless advancing zone. Later variation produced greenish-yellow tints of pyrite yellow, citrine and olive citrine, vivid wax yellow, and brownish shades including deep colonial buff, honey yellow, yellow ocher, raw sienna, and buckthorn brown. Coloration on *potato* was somewhat less intense and varied but within the same range. Aniline yellow and orange citrine took the place of the more greenish tints, and the mats were on the whole less vividly pigmented. On *malt* the color range was very similar to that on potato-dextrose. The filmy growth on *corn* was colorless shadowed with cloudy patches of buckthorn brown and amber to Naples yellow. On *Czapek's synthetic agar* the thin mat was colored amber to barium yellow, with clouds of yellow ocher to buckthorn brown. With age velvety citron yellow patches developed near the inoculum. The agar became deeply stained beneath the darkly-pigmented bands. On *peptone* media staining of the agar was quite common, spreading from the inoculum as centre and blackening the agar in advance of growth. It occurred in tubes in which the beginning of growth was delayed, and was perhaps due to overheating of the inocula used. In all the cultures observed, growth finally spread and was normal in type, presenting on the whole shades from the range above detailed. Pinkish tints, including pinkish buff and wood brown, were introduced in the fluffy growth toward the tops and bases of the tubes. As growth proceeded the blackened agar gradually bleached. On all media bruising of the mats produced a deep brownish discoloration.

Rate of growth. Initial growth was slow, cultures four or five days old presenting often but a thin, narrow halo around the inoculum. Once started, however, the spread was more rapid, and potato-dextrose slants were covered in from two to three weeks. At first the mats on *potato* developed even less rapidly than the above, but slants of that agar also were covered after a period of two to three weeks. *Malt* slants presented somewhat more rapidly spreading mats, and proved the best substratum under observation. The filmy growth on *corn* and *Czapek's synthetic agars* spread rapidly, covering the agar surfaces in less than two weeks. On the *peptone* media considerable variation in the establishment of growth caused marked differences in the extent of the mats, but in all cases the mycelium spread with less rapidity than on potato-dextrose.

Microscopic features. The first formed hyphae were delicate and hyaline, 3-5 μ wide, with clearly-defined but thin walls, frequent branching and septation and no clamp connections (Fig. 69). They continued to form the submerged mycelium, and particularly in

growth in the dark formed a large part of the aerial mat. Often they displayed great irregularity of outline and in the submerged mycelium threads were noted measuring 10-20 μ in width. These often showed marked constriction at the septa (Fig. 70). In old cultures the opposite extreme was found in the not infrequent occurrence of threads 0.5 μ in width. Hyphae of this type remained for the most part unpigmented or slightly yellow in color, but in old cultures many appeared with yellowish or reddish brown coloration.

Within two weeks there was produced an abundance of chlamydospores, the development of which was somewhat more rapid in the dark than in the light. These were produced in terminal or intercalary position, were spherical, subspherical, or elliptical in form, and possessed smooth walls of uniform and moderate thickness. Those of the aerial growth were 6-10 x 8-10 μ in size, but in the submerged mycelium they were found measuring up to 16 x 20 μ. The walls were colorless, and the contents pigmented as the hyphae from which they were produced, ranging from colorless to yellowish or reddish brown.

The vivid coloration of the mats was produced by the development in the aerial growth of beautifully formed, regular hyphae, with smooth, colorless to greenish walls of moderate and uniform thickness and yellowish to reddish brown contents. Under the influence of light, as recorded above, they became massed in tufts (Fig. 67), which standing in close array perpendicular to the agar surface, effected the production of a thick velvet pile. Under other conditions they formed an irregular, much-branched tangle (Fig. 68), interweaving over the surface with the less conspicuous threads of the first formed type. A frequent form of branching in which the developing branches bent abruptly at the point of origin and lay closely appressed to the parent thread is illustrated in Fig. 98. In mats examined during the first month's growth no spore formation was noted in hyphae of this type, but in old cultures they were not infrequent, and were of the type above described usually deeply pigmented. No clamp connections were noted in any culture.

Change in the culture medium produced few hyphal variations. Spores were not found in mats on corn agar, and were very infrequent in Czapek's synthetic agar cultures. On the latter medium slight development of the regular hyphae, typical of aerial growth on other media, occurred. The more irregular hyphae of the first formed type were, however, well-developed, and presented walls slightly thicker than on other substrata.

Examination of the balls and stalk-like outgrowths formed in many cultures revealed no new hyphal variations. A central core of regular pigmented threads was found covered with the velvet pile above described, and in this surface growth the appearance of helicoid hyphae was not infrequent. Many threads were noted, tapering toward the exterior and bearing chlamydospores typical of the species.

(8) *Polyporus sulphureus* Fr.

Hartig (25) in his investigations on oak destruction made a detailed study of the red rot caused by *Polyporus sulphureus*, to which he accorded the rôle of wound parasite. He obtained the fungus also from *Populus alba*, *Pyrus communis*, and *Juglans regia*. More recent observations of Galloway and Woods (23), von Schrenk (45), von Schrenk and Spaulding (47), Atkinson (1), Hedgcock (27), Meinecke (34), and Weir (53) extend the knowledge of its distribution in America to include as hosts practically all our native trees, both coniferous and deciduous.

Material. Cultures here studied were obtained from sporophores and wood of oak, collected at different points in the vicinity of Toronto. Those started in June, 1921, have been carried through seven subcultures and have proved uniform and specifically distinct. Others received from Dr. J. H. Faull and perpetuated by subculturing substantiate the constancy of characteristics. The distinctive and easily recognized mycelium, in respects other than rate and abundance of growth, and slight differences in color, presents characteristics which have proved quite constant on the different media used.

Agars compared: Potato-dextrose, potato, corn, Czapek's synthetic agar and Currie's synthetic agar (modified). Certain characteristics were also noted on Czapek's synthetic (modified) and malt agars.

Habit of growth. On all media the growth, when well developed, was soft, close, light, and downy (Figs. 2 and 7). Differences in cultures depended on differences in amount of growth and rate of advance. There was in all cases a sodden, appressed advancing zone, followed by a light, loose, downy area, and later a gradual thickening to the close, downy, and finally somewhat spongy mat.

Color. The coloration was delicate but characteristic. The different media presented a variety of pale salmon and buff shades, but individual cultures produced quite general and uniform pigmentation. Color on *potato-dextrose* deepened in the first two weeks from white through pale ochraceous buff and pale ochraceous salmon to light ochraceous salmon, but developed no deeper tints in later growth. *Potato* cultures were similar at first, but in the third week

assumed the more orange shade of capucine buff. Cultures on *Czapek's synthetic agar* deepened in tint more rapidly than those on potato-dextrose. At the end of a week there was a uniform coloration of light ochraceous salmon, which in a few days intensified to ochraceous salmon and soon after began to grow dull. *Currie's synthetic (modified)* agar gave the deepest coloration producing a deep capucine buff as the slants became nearly covered. On *corn* the mats remained white to colorless during the first three weeks, and did not deepen beyond a pale ochraceous salmon. All cultures sooner or later lost their bright tints and became a light buff.

Rate of growth. The spread was most rapid on *potato-dextrose*, on which at the end of two weeks the growth was 1.5 cm. in advance of that on *Currie's synthetic medium (modified)* which was second in amount. All the slants, however, except corn were covered at the end of the third week. The advance on *malt* and *Czapek's synthetic (modified)* agars was not measured, but the former presented a final growth even more luxuriant than that obtained on potato-dextrose, and the latter was similar in effect to the other peptone medium. Development on *corn* was extremely poor. During the first week no visible growth appeared, but later a thin, filmy circle developed and spread, until the slants were covered with mats similar in texture to those on other media, but always thinner.

Microscopic features. The initial growth consisted of hyphae of one type. They were rather delicate, variable in width, 3-12 μ, the widest appearing in the advancing zone. They were rich in fine granular, protoplasmic contents, loosely and irregularly branched, frequently septated and often constricted at the septa (Figs. 103 and 104). No clamp connections were noted. In old cultures hyphae up to 22 μ in width were found. In the aerial mycelium on all media there were developed sooner or later a rich growth of chlamydospores. None appeared during the first week's growth, but they began to appear in the second week, and rapidly formed in abundance. They were borne terminally at the ends of short branches, developed in profusion at the ends of side branches (Fig. 99), thus forming sprays, or were produced in series in intercalary position by the differentiation of any vegetative cells (Fig. 100 *a-d*). The terminal spores were subspherical; those, in intercalary position, were very irregular, following often the shape of the cells from which they formed, elliptical, oblong, triangular, etc. They were 6-7 x 6-11 μ in size. In the submerged mycelium, which resembled the aerial except that the walls were thinner, there were produced chlamydospores, also irregular in shape and much larger than those described above (Fig. 102 *a-d*).

They measured 10-13 x 18-30 μ, had smooth, colorless, hyaline, relatively thin walls, and dense, granular contents. These also developed in terminal or intercalary position. In cultures a month old there were noted in the aerial growth specially differentiated, fine hyphae, with thick, colorless, hyaline walls and narrow lumina. These were branched and infrequently septated (Fig. 105), 1-3 μ wide, and originated as specialized branches of the broad hyphae (Fig. 106). The different media presented few special peculiarities. On *Czapek's synthetic* medium no spores developed in the submerged mycelium, while on the *peptone* media spores of both types appeared in the aerial growth. Those ordinarily present only in the submerged layers were, however, infrequent in the aerial. On *corn* terminal sprays of spores were rare, and occurred only in rather old cultures, but the hyphae broke up almost immediately after formation into especially thick-walled spores, very variable in size and shape, and often larger than those on other media, 5-10 x 7-14 μ in size (Fig 101).

(9) *Polystictus abietinus* (Dicks.) Fr.

Polystictus abietinus is found as a destroyer of the sapwood of coniferous trees throughout Europe and America. Its occurrence on dicotyledonous wood is rare, but Weir (54) records finding typical sporophores on *Populus trichocarpa* in the northwest. The only reference noted to its activity in the destruction of living trees is that of Rhoads (41) who in a detailed study of the closely allied species, *Polystictus pargamenus*, concludes that the death of larch trees in Minnesota, reported by Freeman as due to the latter fungus, was in reality the result of the action of *Polystictus abietinus*.

Material. All cultures here considered were made from wood or sporophores collected from dead trunks of *Abies balsamea* or *Picea mariana*. They include material from eight collections, and contain the results of wood and sporophore inoculations from both hosts.

Agars compared: Potato-dextrose, potato, malt, Czapek's synthetic and Czapek's synthetic (modified).

Habit of growth. Early growth on *potato-dextrose* consisted of a close farinaceous mat with broad, sodden, appressed advancing zone. It extended and thickened to form a closely woven, skin-like growth, with a felty-woolly surface, and characterized by deep radiating furrows (Fig. 41). *Czapek's synthetic (modified)* agar produced a mat similar to that on potato-dextrose but thicker. On *malt* the more rapid thickening left a narrower, but still distinct, sodden advancing zone. The mat, at first somewhat silky, became closely woven, and presented a smooth, even, unfurrowed spread. After a month's

growth on potato there was produced only a thin, filmy mat, closely appressed and farinaceous to sodden in appearance. Early development on *Czapek's synthetic* agar was represented by a thin, loosely tangled, downy aerial growth, which with age became matted and dry.

Color. On *potato* and *Czapek's synthetic* agars the mycelium remained white to colorless throughout its growth; on *malt* a faint cream color developed in two weeks, but did not deepen; on *potato-dextrose* and *Czapek's synthetic (modified)* agars the coloration included a shadowing of light to pinkish buff and avellaneous in the older growth, but even the deepest tints were by no means pronounced.

Rate of growth. Growth proceeded with moderate rapidity on *potato-dextrose* and *malt.* On the former the mat extended over about 7 cm. of the slant in the first two weeks, and in less than three weeks the slants were entirely covered; on the latter the advance was slightly more rapid but less compact. *Czapek's synthetic medium* (modified) produced a slowly spreading, but thick and closely woven growth, which in less than a month covered the agar surface. *Potato* and *Czapek's synthetic* agars were unsatisfactory, presenting in the former case farinaceous-filmy, and in the latter light, loose downy mats, often zonated, which eventually covered the slants, but remained thin and poor.

Microscopic features. The growth was characterized on all media by a development of firm-walled, branched, septated, hyaline hyphae, which showed an abundance of prominent clamp connections. On *potato-dextrose* the closely interwoven hyphae of the aerial growth were especially strong walled; showed often, particularly in the older areas, a lack of contents, and tended to send off short upright branches, which produced in mass the soft, felty-woolly surface. These latter were further distinguished by terminal and intercalary swellings (Fig. 87), varied in shape and measuring 8-9 μ in diameter; ordinary hyphae were 3-5 μ in width. Hyphae 2-4 μ in width, similar to those of the ordinary type, but with thinner walls and rich protoplasmic contents formed the submerged layer. On *potato* the growth, which was slight, consisted of hyphae of the ordinary type, the development of which seemed hindered by the deposition of masses of tiny crystals, soluble in hydrochloric acid, which formed encrusting layers, more or less dense, on the majority of the threads. The aerial growth on *malt* was readily distinguished by the abundance of aggregations of tiny crystals forming caps on the ends of short side branches (Fig. 88). These were in the form of spherical or conical clumps, and suggest the crystal crowns found upon the cystidia of this fungus. The short

branches here noted were, however, of uniform diameter, while the similarly capped branches of the hymenial surface are either club-shaped, or terminate after slight enlargement in an abruptly narrowed prolongation, over which the crystals are massed. A few similar branches, bearing crystals, appeared on potato-dextrose and Czapek's synthetic agars, but their occurrence was rare. The latter medium presented hyphae typical in form, but scantily developed in a loose tangle. On the *peptone* medium growth was very compact, but the hyphae were similar to those on potato-dextrose.

(10) *Polystictus versicolor* (L.) Fr.

Polystictus versicolor is probably the most destructive and widely distributed of the wood-destroying fungi which attack dicotyledonous wood. Its activity is manifested chiefly in the destruction of fallen or structural timber, and Bayliss (2) has designated it as a pure saprophyte. Galloway and Woods (23), however, report it as the cause of root rot in many living trees, citing as evidence of disease a stunted growth of wood and an over-production of fruit. Von Schrenk (46) and von Schrenk and Spaulding (47) report its occurrence as a wound parasite of the Hardy Catalpa and discuss its agency in the production of a soft rot of the wood of that tree. It has been found also by the writer as the causal agent of butt rot in living *Fagus americana*. In the tree attacked the fungus had penetrated the wood a short distance above the ground and was proceeding upwards in the trunk and downwards in the roots, in some of which for some distance the heartwood and sapwood were found completely destroyed. The decay advanced centrifugally, the heartwood becoming gradually bleached in the region of fungus penetration and assuming a light creamy coloration, while towards the limits of advance the wood became stained a russet brown. The completely penetrated root was reduced to a brittle but not friable mass, light yellow in color.

Material. Cultures here discussed were obtained from wood and sporophores taken from living *Fagus americana*, and from sporophores from fallen trunks of *Ulmus americana* and *Betula latea*.

Agars compared: Potato-dextrose, potato, corn, malt, Czapek's synthetic, and Czapek's and Currie's synthetic (modified).

Habit of growth. The characteristic mode of growth, presented sooner or later on all media except corn, was a very closely woven, moderately smooth mat of chalky appearance (Fig. 42). After four days' advance on *potato-dextrose* there was produced a close, even, felty-woolly mat, becoming downy toward the upper limits and with growth sufficiently rapid to leave visible but a narrow, appressed,

colorless advancing zone. As growth proceeded the hyphae became very tightly interwoven, forming a thick, compact mat with a surface of characteristic chalky appearance. *Malt* produced a mat very like that on potato-dextrose. Growth on *potato* also was very similar, though less dense. On *corn* there was obtained merely a thin film, at first laden with tiny moisture drops, but presenting later a cloudy, dry surface. The early growth on *Czapek's synthetic* agar was poor, five days' development producing merely a thin, almost invisible, but rapidly spreading film. It thickened irregularly, forming chalky, filmy and colorless, and toward the top, downy bands. In the final development the mats were marked into definite areas, the older growth becoming thick and chalky as on other agars and remaining slightly yellowed, while the upper area showed decided coloration provided by a thin, hard layer at the agar surface. This was lightly and irregularly covered by a thin, dry, matted growth. The mats on the *peptone* media were practically identical, and distinguished from those on potato-dextrose chiefly by their more pronounced coloration.

Color. On *potato-dextrose* the mats remained pure white for nearly two weeks, and throughout the later development a slight yellowing over the surface, and small scattered areas especially toward the base of the tubes, showing old gold staining were the only indication of pigmentation. On *potato* the mat remained white throughout the period of observation, and on *corn* the thin growth continued white to colorless during a month's growth. As the *malt* cultures aged the surface became slightly yellowish. *Czapek's synthetic* agar produced the most pronounced coloration. The filmy growth, gradually becoming zonated with downy and colorless bands, thickened and hardened. The older growth remained white or faintly yellowed with small, greyish olive areas. Toward the top of the tubes the development of a deeply pigmented layer at the surface of the agar cast a greyish olive coloration through the matted downy superficial growth. The *peptone* media presented similar mats. The initial white growth became faintly cream color, later showed shadowings of cartridge buff and massicot yellow, and toward the end of three weeks produced bands of Naples yellow and smoke grey. More advanced growth dimmed the coloration by the production of a dry, superficial layer.

Rate of growth. On all agars spread of the mycelium was rapid, completely covering the slants in twelve days' growth.

Microscopic features. Initial growth on all media presented a profuse development of colorless, hyaline hyphae, 2-6 μ wide, much branched and septated and richly furnished with clamp connections (Fig. 81). These continued to form the advancing zone and sub-

merged mycelium, often showing great expansion and irregularity of outline (Fig. 83). In a few days there was differentiated in the older aerial growth an abundance of fibre-like hyphae. These were colorless and hyaline, with thick walls and narrow lumina, were sparingly branched and septated (Fig. 84) and furnished very rarely with clamp connections (Fig. 85). They were ordinarily 2-3 μ wide, but threads 5 μ in diameter were not infrequent; and they were further characterized by the more or less frequent occurrence of swellings, which were sometimes terminal, but more often intercalary (Fig. 86), and which were formed by slight expansion of the lumina accompanied by local thickening of the walls.

Secondary spores. *Polystictus versicolor* exhibits great tenacity of life, retaining its vitality under the most adverse conditions. Bayliss (2) reports the revival of vegetative and reproductive activity in the mycelium contained in a museum specimen after four years' storage, and the wide and abundant distribution of the organism in nature attests its adaptability to varied conditions. Its powers of secondary spore production doubtless assist in its dissemination and persistence. Patouillard (36) found conidia in connection with sporophores found in the field. Brefeld (7), Bayliss (2), and Lyman (32) record observations on oidial formation upon the primary mycelium in culture, and Bayliss (2) reports also the production of conidia on the secondary mycelium. Their appearance was considered due to unfavorable conditions of nutrition. Certainly a six months' growth in hanging drop cultures did not permit of normal development, but the viability of detached cells after that period attests the powers of resistance of the organism. The conidia when transferred continued growth by a process of budding, or by the production of colorless to olive green oidia. These oidia were obtained in flask and tube cultures on glycerine medium, which owing to the scanty growth produced was considered unfavorable, and on malt gelatine after eight months' development. In all cases they appeared as organs of the secondary mycelium in exhausted or otherwise poorly nourished cultures.

The mycelium here studied yielded none of these types of reproduction. Lack of spore germination studies precluded the possibility of observations upon the organs of the primary mycelium; and a difference in the choice of culture media and of other conditions of growth doubtless account for the non-production of conidia. A third type of secondary spore has, however, been obtained, rarely on most media, but in marked abundance on *Czapek's synthetic agar* (modified). These were chlamydospores (Fig. 82). They occurred in the hyphae bearing clamp connections as modifications of cells terminal or inter-

calary in position. The mature spores were spherical to subspherical, elliptical or irregular in shape, 8-10 x 8-16 μ in size, had smooth, fairly thick, colorless, hyaline walls, and rich, granular, protoplasmic contents. They were first observed in limited numbers in an eight days' growth on Czapek's synthetic agar (modified); at the end of two weeks they were noted as abundant, and their production continued. Cultures two months old yielded a plentiful supply. No spores were noted on corn, and growth on Currie's synthetic agar (modified) was not examined microscopically, but on the other media tested no spores were observed in cultures less than two weeks old and their occurrence was extremely rare in all the mycelium examined.

Other than in the case of spore production, striking individual differences did not characterize growth on the several media tested. A looser interweaving of the hyphae on malt resulted in a mat of softer texture than that on potato-dextrose. Accompanying and rendering possible the rich spore production on Czapek's synthetic agar (modified) was a corresponding profuse development of hyphae of the first formed type, and in comparison with other media a proportionately limited production of fibre-like threads. On Czapek's synthetic agar the colored layer at the agar surface was found due to local staining of the medium.

(11) *Trametes pini* Fr.

Trametes pini causes the most prevalent and strikingly characteristic heartwood rot of conifers known in Europe and America. Advanced decay includes also sapwood destruction. This fungus was carefully studied and described by Hartig (25); and in America its activity has been noted by Atkinson (1), von Schrenk (45), Hedgcock (27), Meinecke (34), and Rankin (40).

Material. The cultures discussed below were obtained from sporophores collected in the vicinity of Toronto, from wood and sporophores from *Picea canadensis* received from northern Ontario, and from rotted wood of *Abies lasiocarpa* collected in Alberta. The peculiarities of the rot in the last two cases at once suggested this prevalent organism, and the characteristic mats obtained in culture left no doubt as to the identity of the fungus concerned. The oldest cultures on hand have been carried since June, 1920, having been carried through fifteen subcultures, and inoculations from material of subsequent collections produced practically identical mats.

Agars compared: Potato-dextrose, potato, corn, malt, Czapek's synthetic, and Czapek's and Currie's synthetic (modified).

Habit of growth. Early growth on *potato-dextrose* produced a soft, downy ball over the inoculum, with a spreading circle of mycelium, which formed a wide, appressed, colorless advancing zone. The mat spread and thickened, presenting in the older growth a rough, tufted surface, surrounded by a loose, woolly area which passed into the appressed circle at the limits of growth (Fig. 31). The deeper aerial layers became closely woven, producing a thick, but soft mat, which stained deeply on being bruised. The surface layer remained soft and loose, but dried down and dulled as the culture aged. Cultures on *Czapek's synthetic* agar were similar to those on potato-dextrose but much poorer in development. In all cases the pigmentation was more delicate but in some it was but a shadowing over the white mat. On *potato*, growth remained thin. In four days instead of the thick, downy ball produced on potato-dextrose there was but a thin covering, which did not conceal the inoculum. In Fig. 32 is shown the close, soft, woolly mat produced in eleven days. Other series produced coloration more closely resembling mats on potato-dextrose, but in all cases the amount of growth was less. Mats on *malt* were similar to those on potato-dextrose, but softer, less variegated and somewhat downy. On the *peptone* media, which gave practically identical mats, the advancing zone was narrow and the whole growth compact and firmly woven. The surface at first soft, smooth, closely woven and felty roughened later by the development of a tangled, woolly overgrowth. *Corn* produced merely thin, appressed, filmy, white to colorless mats.

Color. The coloration on *potato-dextrose* was vivid and distinguished the fungus at once from *Fomes igniarius*, to the texture and habit of growth of which it most nearly approaches. The advancing zone was colorless and was followed by a creamy white area. Near the inoculum a four days' growth had already assumed tints of honey and mustard yellow. Until the slants were covered progressive changes occurred corresponding to the increasing growth. These changes added tints of old gold, amber yellow, aniline yellow, orange citrine, buckthorn brown, tawny olive and clay color, the deeper tints appearing in the older and in the deeper growth and the lighter toward the limits and in the superficial layers. The mat presented a variegated appearance, fairly well represented in Fig. 31. As the cultures aged the colors dimmed with the drying of the mycelium, and the mats assumed a more even tone of yellowish brown. The superficial layer of agar became browned in old cultures. *Omission of dextrose* diminished the pigmentation in a marked degree. In some potato tubes (Fig. 32) the mats remained white shadowed with Naples

yellow. In other cultures, however, a variegation including yellow ocher, primuline and mustard yellow was obtained; but in no case did the intensity of color equal that on potato-dextrose. Some cultures on *Czapek's synthetic* agar presented, as in Fig. 34, a variegation less vivid and less rapid in development than that obtained on potato-dextrose, but including old gold, chamois, Naples yellow, and cinnamon buff; others, however, remained white shadowed with Naples yellow to chamois. These cultures on potato and Czapek's synthetic agar presented one of the few irregularities in constancy of development, but no explanation can be offered since the media used in different subcultures was made in the same manner, as far as known, and, in addition, proved satisfactory for other organisms. Also *Trametes pini* on potato-dextrose proved invariable. The two *peptone* media yielded practically identical growth. From old gold, amber yellow, and white (Fig. 35) the color deepened to honey yellow and chamois, and later included Naples and barium yellow, vinaceous drab, and cinnamon buff. The agar in the first few days became yellowed, at the end of three weeks was stained a vinaceous drab, and before the end of two months was almost black. On *corn* the mycelium remained white to colorless. The final coloration on *malt* resembled that on potato-dextrose, but the developing mat presented less vivid variegation.

Rate of growth. *Potato-dextrose* and *malt* produced the most abundant growth. Both media proved highly satisfactory and presented strikingly characteristic mats. The mycelium, however, spread slowly: it took over three weeks to cover the potato-dextrose slants. In four weeks the *Czapek's synthetic* agar surfaces were covered, but the growth remained thin; at that time the mycelium was still spreading on *potato* and *malt*, and eventually covered the slants; but in tubes of *Czapek's synthetic agar (modified)*, although the mats were thick, they were so compact and slow in advance that the agar dried out before the surfaces were covered.

Microscopic features. On *potato-dextrose* the first formed hyphae were regular, branched, septated threads, with thin, colorless, hyaline walls and dense, granular protoplasmic contents (Fig. 62). These penetrated the agar and spread over the surface, forming throughout, the elements of the advancing zones in both regions; they continued to form the bulk of the submerged growth and were found in more limited numbers in the aerial mats of all cultures examined. From them were differentiated hyphae with more marked characteristics. In the aerial mat were developed firm, relatively thick-walled hyphae, which usually showed frequent septation (Figs. 59 *c* and 60). The

walls were of uniform thickness, hyaline and colorless, or with a slightly greenish tint. Cross walls were often arched. The contents were colorless to greenish or yellowish brown. They were very regular, much or little branched according to their position, and showed frequent expansions along their course, which were either single or in series and in terminal or intercalary position (Fig. 59 *a*). In the submerged growth these gave rise to a closely woven layer just below the agar surface; and in their development they effected the browning of the adjacent agar. The staining, however, remained localized. The threads of this layer were very frequently septated (Fig. 60), much branched, and closely intertwined. Many cells became empty as the cultures aged, and the contents were never as conspicuous as those of the earlier formed threads. In the aerial growth similar but less frequently branched hyphae gave rise to coarse fibre-like threads, with firm walls of uniform thickness and with septation of varied frequency but showing often no cross walls for considerable distances. The walls were thick, refractive and hyaline, and the contents varying shades of yellowish to dark brown. Expansions of irregular shape occurred along their course as on the earlier formed threads (Fig. 59 *d*). Two or more hyphae of this type were frequently found closely twined together for long distances. They were capable after considerable extension of producing threads similar to those from which they originated. Branching took place in all parts, but was least frequent in the later formed elements. Hyphae usually forked, but very often two opposite branches originated at the distal end of an intercalary cell. Anastomosis was not infrequent, especially in the less differentiated threads. No clamp connections were noted in threads of any type. On potato-dextrose all types of hypha were found at the end of a week's growth, but in older cultures a greater proportion of heavily-walled threads was noted and expansions were more frequent.

On *potato* agar the first week's growth contained a few pigmented threads, but not enough to give color when viewed macroscopically. Later all the elements which developed on potato-dextrose were found, but the coarser hyphae were always less abundant. No closely woven submerged layer was formed. *Malt* cultures closely resembled those on potato-dextrose, but continued to show in the aerial mat a greater proportion of hyphae of the first formed type. All the elements noted on potato-dextrose were found less abundantly developed on *Czapek's synthetic* agar. Growth on *Czapek's synthetic agar (modified)* began with the formation of simple, hyaline hyphae similar to those above described, and these were found throughout

the mat. An extremely compact layer was formed just below the agar surface with the threads cemented together so firmly that they gave the appearance of a solid tissue. These showed frequent septation and many spherical and irregular expansions. The contents of the hyphae were colorless, yellowish or dark brown, as were those of the aerial mat. The agar, as before noted, was deeply stained. In the aerial mat the deeper layer was closely woven, and the superficial growth formed of loosely branched threads, which tended to grow perpendicular to the surface. These branched by forking or by the formation of opposite branches. The hyphae were beset with innumerable, hemispherical, sac-like expansions (Fig. 61) which distinguished the mycelium from that on any other medium. No long, fibre-like threads were formed. Pigmentation ranged from light yellowish to dark reddish brown.

Rots of Abies balsamea

The prevalent balsam rots, which I have had the opportunity to examine, may be grouped under three types as illustrated in Figs. 51-54. The classification of these rots employed corresponds to that used by Faull in a report on Forest Pathology in the report of the Minister of Lands and Forests, Province of Ontario, 1922, as follows: Types A and C are Types I and IV respectively, and Type B includes Types II and III. These, with the organisms isolated from each, are considered below.

Type A (Fig. 54). This type of rot is in macroscopic characteristics similar to that ascribed by Hartig (25) to the action of *Polyporus mollis*, and later (26) identified by him as the result of the attack of *Polyporus schweinitzii*. In early stages it causes a yellowing of the heartwood, and with advancing decay the coloration deepens through light to dark brown. The wood gradually dries and, with the consequent contraction, cracks along rays and annual rings, breaking the heartwood into more or less cubical blocks composed of a decayed residue, which is very light in weight, charcoal-like in appearance, and extremely friable. Sheets or pads of greyish mycelium usually fill the spaces left by the contracted wood. A longitudinal view of wood thus decayed is shown in Fig. 54. From such wood two organisms have been obtained, which possess distinctly characteristic mycelia. The first has been identified as *Polyporus balsameus*, since it presents in culture the typical growth produced by fruit body tissue cultures of that species; the second remains unidentified, corresponding to no sporophore culture yet obtained. While found in frequent

association with this type of decay, it seems very possible that the action of the latter fungus may be of a secondary character.

(12) *Polyporus balsameus* Peck. (Balsam rot Type A, Fungus 1).

Polyporus balsameus has been recorded by Faull (21) as the cause of heart rot in *Abies balsamea* in the Timagami Forest Reserve. In the same report its probable agency in the decay of cedar is noted. Cultural results confirm the findings in the case of *Abies balsamea*. Mycelium isolated and cultured from rotted heartwood of living balsam entirely agrees with that produced by inocula obtained from sporophore tissue of the above named fungus.

Material. Cultures were first obtained in September, 1920, from decaying heartwood of living *Abies balsamea*. At the same time a similar growth was produced from inocula taken from a fallen balsam trunk, which the previous year had been noted bearing sporophores of Polyporus balsameus. During the spring and summer of 1921 cultures were started from similarly decayed wood of other living balsam trees, and in September sporophore tissue cultures received from Dr. J. H. Faull left no doubt as to the identity of the mycelium previously examined. The sporophores from which inocula were obtained were taken from decaying *Picea mariana* and *Abies balsamea*.

Media compared: Potato-dextrose, potato, malt, Czapek's synthetic and Czapek's synthetic (modified).

Habit of growth. A culture after one week's growth on potato-dextrose presented a mat with straggling, appressed, colorless advancing zone followed by a thin, downy-hairy growth, which tended to become farinaceous in the vicinity of the inoculum. The thickening mat became close woolly to light downy toward the top and the farinaceous area gradually spread (Fig. 26) until in old cultures the entire surface was farinaceous to spongy in appearance. *Omission of dextrose* produced a mat similar in type but much thinner (Fig. 27). On *malt* the more rapid growth left visible no appressed advancing zone; the limits of growth were at first of regularly radiating, silky threads and the mat thick and somewhat downy. The soft, silky to downy texture was retained for some time (Fig. 28), but the farinaceous-spongy appearance due to developing chlamydospores later produced a surface practically identical with that on potato-dextrose. Early growth on *Czapek's synthetic* agar closely resembled that on potato-dextrose. Close, light, downy patches then appeared over the surface for some days and were often covered with brown

moisture drops (Fig. 29). But with age the entire surface became farinaceous-spongy as on potato-dextrose. On *Czapek's synthetic medium (modified)* (Fig. 30) growth was very similar to that on malt, but somewhat thicker especially near the inoculum, where the thick, soft ball was after three weeks' growth laden with moisture. Old cultures presented a matted downy rather than spongy surface.

Color. On *potato-dextrose* a week's growth was insufficient to conceal the agar, but as it thickened the mat appeared white and then gradually assumed brownish tints. Color spread and deepened slowly through cream buff and cinnamon buff, and in old cultures became an even drab with occasional honey yellow patches. Often in cultures a month old white areas of considerable extent were still present, but occasionally the entire surface became brown. On *potato* the mat throughout remained so thin that the agar showing through lent a greyish appearance, but the mycelium was white shadowed with light, yellowish brown. *Malt* and *Czapek's synthetic agar* cultures were practically identical with those on potato-dextrose. On *Czapek's synthetic agar (modified)* the coloration developed more rapidly and deeply, especially in the vicinity of the inoculum, but was similar in character. The agar became stained almost black.

Rate of growth. The mycelium spread with moderate rapidity on all agars, the slants being covered in all cases at the end of three weeks. Initial growth was poorest on *Czapek's synthetic agar* but *potato* was in the end the least satisfactory medium since the mats remained thin throughout. *Malt* and *Czapek's synthetic medium* (modified) produced the most profuse growth and, while spread on the latter was slightly slower, the difference in rate was not marked as in the case of so many species. *Potato-dextrose* gave a growth as rapid as that on malt and practically as satisfactory.

Variations. Old cultures from different sources were more nearly identical than those examined during the first three weeks' growth. Difference in rate of development of the typical chlamydospores, which in mass produce the spongy, brown appearance of the aerial mat, permitted a slight range of variation in texture and color distribution in the earlier growth.

Microscopic features. All media produced mats presenting similar hyphal variations. Initial growth consisted of delicate, colorless, hyaline hyphae, 2-6 μ wide, of firm appearance, branched and septated, and bearing numerous clamp connections. These formed both submerged and aerial layers. Within a week the older growth contained

numerous spores, and even in the advancing zone a few were noted in the process of development. These were of the chlamydospore type with thick, smooth, hyaline walls, were formed in both aerial and submerged layers by the segmentation of fine hyphae usually about 2 μ wide, and in terminal or intercalary positions, producing in the first case conidia-like sprays and in the latter long, bead-like chains. The majority were elliptical to sub-spherical in shape, and 10 x 12 μ in size; but they often varied in form with the segment of the mycelium going to their formation, and ranged 7-10 x 12-22 μ in size (Figs. 73 *a-e* and 74). In the submerged layers the spores were on the whole colorless or faintly yellow, but on Czapek's synthetic agar (modified) they were pigmented as in the aerial growth. The walls in all cases remained colorless, and with the rounding off of the spores secondary walls were frequently formed. The contents varied from colorless through yellowish to deep brown, and were arranged somewhat concentrically. One or two globular masses filled the centre of the spore and were surrounded by successive thin and dense layers.

As the mat developed some of the hyphae while similar in type became rather coarser, developing thicker walls and dense dark brown contents. The clamp connections, in all cases well defined, were specially prominent. The clamp septum, usually terminal in position, frequently developed toward the middle of the connecting branch (Fig. 76 *a*). In all cultures also, in both submerged and aerial layers the hyphae formed large, conspicuous, irregular expansions, arranged singly or in series (Figs. 75 *a* and *b*). In the superficial growth these were usually filled with dense, dark granular contents. They were more frequent in the submerged growth and usually developed in series. In very old cultures practically the entire mat formed a powdery mass of spores.

(13) *Fungus* sp. (Balsam rot Type A, Fungus 2).

Material. Cultures have been obtained from wood in various stages of decay taken from sixteen living trees of *Abies balsamea* and from one of *Picea mariana*. The first mycelium obtained has been perpetuated through eleven subcultures from September, 1920, and others were added as material was obtained. Young, actively growing cultures are most easily recognized; older mats with individual variations in their dull, irregular staining, and gradations from downy to felty texture may not appear so markedly uniform, but are easily grouped, and microscopic examination makes confusion impossible.

Media compared: Potato-dextrose, potato, malt, Czapek's synthetic and Czapek's synthetic (modified).

Habit of growth. On *potato-dextrose* (Fig. 4) development was rapid so that no appressed, advancing zone was formed, but the spreading hyphae produced a semi-appressed, silky growth, in which radiating strands were more or less prominent. In nine days the older growth had become closely woven, and presented a felty-woolly surface; the strands had thickened; and the advancing zone remained soft and silky. With age the mat became more tightly felted, formed at the base a close, thick mat with more or less warted felt, and toward the top a smoother, skin or crust-like layer covered with a matted tangle of long, loose hyphae (Fig. 5). *Potato* agar yielded a growth, similar to the above but much thinner. The radiating strands were much more distinct, and in cultures a month old deposits of colorless moisture were noticeable over the surface. *Malt* cultures resembled those on potato-dextrose very closely, although the strands were less pronounced, and the initial growth more downy in texture; and in older cultures the darkened, skin-like undergrowth of the aerial mat extended farther down the slant. Early growth on *Czapek's synthetic* agar was thin and filmy, with faint strands, which thickened more rapidly than the surrounding mat and formed a somewhat dense network through the aerial growth. With age the surface layer became somewhat woolly and dotted with soft balls among the strands. This later became somewhat warted and covered with a tangled overgrowth, but the mat remained always thin with filmy areas. Substitution of *peptone* for nitrate changed the mode of growth. The mat after five days was thick, even, appressed, and felty-silky with fine radiating strands. It remained skin-like, contracted to form radiating furrows and formed an overgrowth of soft, even, felty texture.

Color. The early growth on *potato-dextrose* was white to faintly cream color. It soon became shadowed with massicot yellow and the strands assumed the same tint. In two weeks there was a noticeable browning of the lower surface layer toward the top of the slants. This area became almost buckthorn brown with a light downy to tangled, yellow overgrowth. Older cultures were variegated baryta yellow and white, with extended areas browned as above noted. *Lack of dextrose* produced a mat less deeply tinted. In two weeks it was mostly white with faintly yellowed strands, and older cultures were less decidedly yellowed and presented no deeply browned layer. On *malt*, the coloration was similar to that on potato-dextrose: cream color deepening to baryta yellow, with buckthorn brown staining

toward the top. The agar was stained brown, the superficial layers very deeply and the lower layers less distinctly. Growth on *Czapek's synthetic agar* remained white during the first two weeks. Later the strands deepened to Saccardo's umber and the surface growth was variegated white and baryta to massicot yellow. On *Czapek's synthetic medium (modified)* the initial growth was white to cream color. Patches soon deepened to massicot yellow, and the agar gradually became stained almost black. As growth advanced the thin, rough felt became buffy brown, with an overgrowth of pinkish buff to cream color, which gradually became brown.

Rate of growth. Advance was rapid especially on *potato-dextrose* and *malt*, on which the slants were covered in ten days. On *potato* and *Czapek's synthetic* agars the mats had extended to the tops of the agar surfaces in two weeks. Czapek's synthetic agar (modified) was the only medium which did not promote a rapid spread and on this, even at the end of a month, the slants were but half covered.

Variations. In cultures from different sources the initial semi-appressed, silky growth presented few variations. In cultures a week old slight differences in the rate of spread, in the prominence of the strands, and in the intensity of coloration distinguished individual organisms. They also varied in texture, some remaining light and fluffy, while others became more densely felted. But in all, the rapid spread, the silky advancing hyphae, the more or less distinct strands, and the delicate yellow tinting were unmistakable. The most striking variation in older cultures was the somewhat rare occurrence of growth without the production of deeply browned layers. Other differences in color intensity were slight, including only a range of pale yellow shades. Variations in texture included the production of surfaces felted in irregular patches or warted, or of mats which remained downy-woolly or downy-felty. In all, the more or less tangled overgrowth was present; and around the sides of the tubes extended anastomosing yellow strands.

Microscopic features. Mats growing on *potato-dextrose* presented at the end of nine days' growth the following elements. The bulk of the mycelium, both submerged and aerial, consisted of delicate, hyaline hyphae, 2-8 μ wide, which were sparingly septated, branched usually at right angles, and filled with granular contents. The walls were of uniform thickness but thin. In the aerial growth were fine, hyaline, fibre-like threads, 1-2 μ wide, with comparatively thick walls and narrow lumina. These were branched, often at right angles and as far as noted were unseptated. Both types lacked clamp connections. The most striking and characteristic microscopic feature

observed was presented by the broad irregular hyphae 10-12 μ wide, which traversed the mat. These possessed thin, readily collapsible walls, were septated and branched, and rich in protoplasmic contents. Constriction at the septa was frequent and many septa were surrounded by conspicuous clamp connections (Fig. 97 *a-e*). These were single, paired or whorled. Ordinary branches occasionally replaced one or more of the encircling clamps, and in other cases branching from a clamping hypha occurred. The threads were mostly hyaline; but in some, dark yellowish brown contents were present. In old cultures the aerial mats were formed chiefly of extremely fine hyphae 0.5-3 μ in width, of which the majority were of the type first described. All types, however, were present, although in very old cultures clamp connections were difficult to find. The elements on *potato*, *malt*, and *Czapek's synthetic* agars were similar, except that on potato, fibre-like threads were not found. They were infrequent on malt but very abundant on Czapek's synthetic agar. On the *peptone* medium the submerged mycelium resembled that on other media, but contained yellowish brown contents. Hyphae bearing clamp connections were also pigmented. Just at the agar surface was formed a brittle layer of firm, deeply pigmented hyphae, very closely interwoven and giving rise to gnarled threads, which interlaced over the surface.

Possible relationship. Certain peculiarities both macroscopic and microscopic suggest the description of cultures of *Coniophora cerebella* made by Möller (35), and again by Rumbold (42). The former used spore cultures on a substratum of sawdust dispersed in malt agar, and obtained a mat described in the words: "Neigung zu strangartiger Verflechtung der Fäden tritt hervor. Im weiterem Verlauf nimmt das Mycel eine deutlich lehmgelbe Farbe an;" and, "Die Oberfläche war stellenweise mit den rein weissen Oidienmassen bedeckt, andere Stellen zeigten das Lehmgelb junger Fruchtkörper, wieder andere in einer Ausdehnung von mehreren Centimetern eine glatte Oberfläche mit der braunen Farbe reifer Fruchtkörper." In cultures from balsam here discussed no oidia were noted, but the interweaving to form strands, and the coloration of yellow and later brownish tints were found characteristic. Microscopic examination, as above noted, revealed also the whorled clamp connections, which according to Möller, "ausserordentlich auffällig sind, und das Coniophoramycel in bestimmtester Weise charakterisieren." Rumbold's cultures agreed in coloration, oidial formation, and clamp connections with those of Möller. Falck (18) cultured *Coniophora cerebella*, *C. merulioides*, *C. cystiophora*, and *C. brunnea*, found specific mycelial peculiarities of diagnostic value, but noted whorled clamp connections in all four.

He later (19) obtained sporophore production by *Coniophora cerebella* in pure culture.

Burt's report (13) on the Thelephoraceae of North America records all our species of Coniophora as saprophytic on wood and the cause of dry rot, and *Coniophora cerebella* as widely distributed throughout the northern United States and Canada. Should the mycelium here considered prove to belong to this fungus or to an allied form its relationship to living trees, whether primary and parasitic or secondary and saprophytic, will remain to be determined.

Type B (Figs. 51 and 52). This rot agrees with that assigned by von Schrenk (45) to the action of *Poria subacida*. The invaded wood shows until rather an advanced stage of decay numerous black specks, up to 0.5 x 4 mm. in size, which act as centres of hyphal penetration. They are surrounded by white mycelium which gradually spreads tangentially in annual rings often causing marked lamination. Radial penetration from these centres frequently results in numerous perforations of the dissociated layers. The wood becomes yellow and more or less wet, and in advanced stages further disintegration of the wood layers leaves the centre of the trunk hollow or filled with a wet, fibrous residue, fragments of which when dried are easily powdered between the fingers.

The cultures started from twenty-five blocks of wood decayed in this manner, fell into three groups. Eight of them agreed with sporophore tissue cultures of *Poria subacida*; eleven showed the type of growth detailed as Type B (2), and six that described under Type B (3). In blocks from which cultures were started lamination was more or less marked; the central portion remained sound, became laminated, shredded, or fell away; radial perforations were conspicuous or absent; but no particular character seemed associated with any organism with sufficient certainty to make possible a sure diagnosis without resorting to cultural methods. The cultural studies here reported, however, can lay no claim to settling the identification of organisms associated with this rot. The three types of mycelium described below have been found distinct from one another, but the first only corresponds to sporophore cultures thus far obtained; and in all cases the possibility of the isolation of secondary organisms must be borne in mind.

(14) *Poria subacida* (Balsam rot Type B, Fungus 1).

Material. The first cultures obtained were started from decayed wood of living *Abies balsamea* in May, 1920. Seven subsequent

isolations from similarly rotted wood have been found to agree with these, and with fruit body tissue cultures made from sporophores of *Poria subacida* obtained from fallen trunks of *Abies balsamea* and *Picea mariana*.

Agars compared: Potato-dextrose, potato, malt, Czapek's synthetic and Czapek's synthetic (modified).

Habit of growth. On all media used the growth was very similar in appearance (Fig. 44). The developing mat was thin, light and furry to flocculent, with appressed, colorless advancing zone. The latter in early growth on *potato* media was broad and even, formed of long, parallel threads; but, as the mycelium spread, the rate of growth increased and tended to conceal the surface layer. With age the mat thickened, becoming close downy, somewhat flocculent, and in old cultures woolly and matted. At the top the thick, loose, downy texture gave place to a dry, firm rim around the edge of the agar. On *malt* the mat was from the beginning somewhat thicker and the appressed advancing zone narrow and inconspicuous, but the type of growth was exactly similar to that on potato-dextrose. The mat on *Czapek's synthetic medium* was thin, appressed filmy to furry, and somewhat looser and more open than on other media; and through it, after two weeks' growth, anastomosing strands formed a fine network. The *peptone* medium produced a more compact mat, woven in old cultures almost to a felty texture, but similar in other respects to growth on potato-dextrose.

Color. The mats on all media remained pure white during the first ten days' growth. Sooner or later, however, all became slightly discolored by a yellowing, which appeared first at the top, but eventually spread somewhat irregularly over the surface. Toward the end of the second week cultures on *Czapek's synthetic agar* showed a slight staining, and a week later rims of yellow were noted at the upper edges of the mats on *potato* media, but on *malt* and *Czapek's synthetic (modified) agars* no discoloration appeared until much later, when the cultures were drying out.

Rate of growth. The mycelium spread rather rapidly on all media, the slants being covered in two weeks except in the case of the *peptone* medium, on which advance was somewhat less rapid. Slants of this latter were, however, covered within three weeks.

Microscopic features. Initial growth consisted in all cases of delicate, hyaline hyphae, 2-6 μ wide, with walls of moderate and uniform thickness, and coarse, granular contents. These were much branched and septated, and furnished with an abundance of clamp connections. They formed the submerged layers throughout the

course of development and the advancing surface growth; and were present, although in small numbers, in the older areas. Aerial mats of a few days' development showed an abundance of fibre-like hyphae 1-3 μ wide, with thick, refractive walls and narrow lumina. These were sparingly branched and unseptated, furnished in young cultures with granular contents but soon becoming empty. They formed the bulk of the aerial growth in old cultures. Heavy deposits of crystals were found encrusting many submerged hyphae on *Czapek's synthetic* agar; but the different media presented no marked hyphal variations.

(15) *Fungus* sp. (Balsam rot Type B, Fungus 2).

Material. The first cultures obtained were set up in May, 1920, inocula having been taken from decayed wood of *Abies balsamea.* Ten other isolations made subsequently from rotted *Picea mariana* and *Abies balsamea* have yielded growth of similar type.

Agars compared: Potato-dextrose, potato, malt, Czapek's synthetic and Czapek's synthetic (modified).

Habit of growth. Early growth on *potato-dextrose* produced a soft, silky ball over the inoculum. As the mycelium spread the long silky threads tightened down and matted in the older growth, but the advancing zone remained light silky to silky-downy. By the end of two weeks yellow moisture appeared on the surface, usually in bands some distance above, but occasionally in the vicinity of the inoculum. The silky appearance soon passed to a downy or downy-woolly tangle, and when the slants were covered the fluffy growth dried down and finally produced a dry, thin, smooth, soft mat of delicate texture. Development on *potato* and on *malt* was slightly more profuse than on the above, but was exactly similar in type. On *malt* a greater and more widely distributed exudation of moisture was noted. Growth in *Czapek's synthetic agar* was poor, the ball over the inoculum being represented by but a few matted threads; and during a month's growth there was produced but a thin, matted downy to downy spread. The *peptone* medium produced a mat very similar to that on malt, but somewhat slower in development, thicker and more compact in form.

Color. The first growth was white on all media; but by the end of a week yellow moisture drops were noted on the surface of all cultures except those on Czapek's synthetic agar. On that medium the excretion of moisture was delayed until the third week, and was at all times small in amount. At first, coloration was limited to the vicinity of the moisture, and included tints of pinkish, warm, and light buff; but later, when the excretion dried, these patches became overgrown

with a white superficial layer; and in old cultures the color was restricted to the upper part of the slants, and was cream buff fading off to the white of the lower growth. Coloration was similar but more extended on the *peptone* medium, pigmentation in old cultures covering the entire surface.

Rate of growth. Spread of the mycelium took place at a rather rapid rate, agar slants of malt and potato being covered in two weeks. On *potato-dextrose* and *Czapek's synthetic* media the mats advanced with less rapidity, and on *Czapek's synthetic medium (modified)* mats were even slower in spreading; but all the agar surfaces were covered by the end of the third week.

Microscopic features. The first formed hyphae were strong, colorless, hyaline, septated, much-branched, and furnished abundantly with clamp connections. They were 2-6 μ wide and often showed considerable irregularity of outline. The walls were of moderate and uniform thickness and the contents dense and granular. These threads formed the bulk of both aerial and submerged layers on all media. In the yellowed areas they were faintly to decidedly pigmented. On *malt* by the end of two weeks, and on *potato-dextrose*, *potato*, and *Czapek's synthetic* agars before the end of a month there developed a type of fibre-like threads. These were fine, 1-2 μ wide, with comparatively thick walls and narrow lumina, were unseptated, and profusely branched. They were found in the aerial mats on all media except Czapek's synthetic agar (modified), but never in such abundance as to form the predominating element.

(16) *Fungus* sp. (Balsam rot Type B, Fungus 3).

Material. The organism here described has been isolated from blocks of decayed wood obtained from six different, living trees of *Abies balsamea*. All the cultures at hand which fall into this series were obtained during the summer of 1921. When started they were, owing to similarity of sources, grouped with cultures of *Poria subacida* and *Type B* (*2*), but the habit of growth soon enabled the separation of a distinct type, which has proved constant.

Agars compared: Potato-dextrose and malt.

Habit of growth. The early growth on potato-dextrose was thin, farinaceous-filmy to matted hairy with a rather regular, appressed, colorless advancing zone. The developing mat became felty-woolly, quite smooth and evenly woven, presenting at first a somewhat rough, hairy-woolly surface, which with age dried down considerably. On *malt* the habit was quite different. Growth radiated from a downy to hairy ball over the inoculum and spread rather rapidly. Around

the inoculum a dried, matted circle soon appeared, beyond which developed a thick, close, soft, downy growth, more or less covered with moisture drops. At the limits of growth decided silkiness was evident. Old mats presented the same features: very soft, but abundant close downy growth, with a tendency to silkiness toward the edges.

Color. On *potato-dextrose* the mat, white at first, gradually assumed a delicate ivory yellow coloration. The growth on *malt* remained white during the first month, and only in old cultures showed final yellowing toward the top.

Rate of growth. On *malt* the mycelium spread rather rapidly, covering the agar surfaces in about two weeks; but on *potato-dextrose* the more closely woven mats did not reach the top of the slants until more than a week later.

Microscopic features. The advancing hyphae were delicate, colorless, turgid, hyaline threads, 2-5 μ in width, with rather thin, hyaline walls, and finely granular contents. They were branched, septated, and furnished abundantly with clamp connections. The one type slightly modified in different regions provided all the variations noted on both media. In the closely woven aerial mat on potato-dextrose the threads were quite uniform, 0.5-3 μ (mostly 2 μ) in width, for the most part devoid of contents, with clearly defined walls of moderate thickness and abundant clamp connections. In the submerged layers similar threads, 1-3 μ wide, formed a very dense mat just below the agar surface; the deeper growth was loose and not abundant, but formed of similar elements. In young mats hyphae up to 7 μ in width were noted. On malt the downy growth showed loosely branched threads, 2-7 μ wide, many of which were empty in old cultures. In this area helicoid hyphae were not infrequent. Near the inoculum there was a greater proportion of turgid threads, 1-2 μ in width. Scattered throughout the mycelium were numerous bunches of small crystals. Beneath the somewhat loosely woven top layer in the submerged growth threads, penetrating at right angles to the surface, formed a somewhat conspicuous feature. These were regular, fine, mostly 2 μ wide, and filled with granular contents. They terminated at a somewhat uniform depth. Beneath these was a loose interweaving of hyphae 2-4 μ in width, with coarsely granular, refractive contents and less healthy appearance.

Type C (Fig. 53). Heartwood destroyed in this manner becomes buff pink to brownish in color. Infection spreads somewhat irregularly leaving unstained patches surrounded by discolored tissues, which

give a somewhat mottled appearance to transverse sections (Fig. 53). Distributed irregularly over longitudinal faces are small, scattered sheets or pads of white mycelium where the rot is well advanced. The fungus advances irregularly, the margin of the affected area having an undulating to pointed circumference as seen in cross section. From the several pieces of rotted balsam showing this type of decay, from which isolations were attempted, but one organism has been obtained; and it corresponds to no sporophore culture yet studied.*

(17) *Fungus* sp. (Balsam rot Type C, Fungus 1).

Material. Rotted wood has been received from the province of Quebec, where in certain localities this is reported as the most prevalent type of decay, and from the Timagami Forest Reserve, Ontario. In all it has been isolated from six pieces of wood of *Abies balsamea.*

Agars compared: Potato-dextrose, potato, malt, Czapek's synthetic and Czapek's synthetic (modified).

Habit of growth (Fig. 43). Initial growth on each medium was soft, shaggy-downy, with a thick, shaggy ball over the inoculum except in the case of Czapek's synthetic agar, on which development was less profuse. The advancing zone was colorless, semi-appressed, and regular in its advance. After three weeks' growth on *potato-dextrose* the snow-white mat was thick, soft, and downy-woolly. The *omission of dextrose* produced a much thinner, light tangled mat, dotted with tiny, colorless drops of moisture. Early growth was densest on *malt,* but later development more profuse on *potato-dextrose* and *Czapek's synthetic (modified) agars.* All three media, however, proved satisfactory. On *malt,* the mat became tangled-downy and laden with large moisture drops. Growth on *Czapek's synthetic* agar remained thin, tangled and dry until the end of a month, but moisture finally appeared. The substitution of *peptone* for nitrate induced a growth very similar to that on potato-dextrose, but the mat was more tightly woven. Near the inoculum growth was soft, downy-felty, but toward the top it became sodden. Excreted moisture lay in brownish drops over the surface, and spreading in old cultures rendered the entire mat sodden in appearance. In all cultures excretion of moisture increased with development and the underlying mycelium became matted and somewhat appressed.

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*I am permitted to mention that since this was written, field studies by Dr. J. H. Faull have shown *Stereum sanguinolentum* to be the casual agent of destruction in this type of decay; and in addition, cultures of the organism described above, made by Miss I. Mounce on wood have resulted in the production of fruit bodies of that fungus.

In old cultures on *potato-dextrose* and *malt* growth near the inoculum became felted and often formed circular ridges surrounding saucer-like depressions, the floor or sides of which became dotted with tiny moisture drops, which dried leaving pore-like pits. In one culture this felted area took the form of a shelf, suggesting the development of a bracket-shaped sporophore. It was 1 x 0.5 x 0.5 cm. in size, with an upper smooth, felty surface tinted light buff to vinaceous buff, and with felted, slightly woolly lower surface. No hymenial formation was noted.

Color. Throughout a month's development growth on *potato* and *malt* remained pure white, while on *potato-dextrose,* toward the end of that period the mat became very faintly cream color near the inoculum, or, in some cases, produced conspicuous patches of baryta yellow. On *Czapek's synthetic* agar a light yellow coloration developed during the fourth week, and in old cultures a shadowing of massicot yellow was noticeable spreading over an underlying white mycelium. *Czapek's synthetic agar (modified)* presented a very pale ochraceous salmon tinting after three weeks' growth, and this pinkish coloration slightly accentuated, persisted until the increasing brownish excretion covered and rendered sodden the entire surface. The agar in these tubes also browned, becoming at last mummy brown in color.

Rate of growth. Advance was rapid particularly on *potato-dextrose, potato,* and *malt,* on which the agar slants were covered in ten days. On the *synthetic* media spread was slightly more retarded, but even on these the agar surfaces were almost covered by the end of two weeks.

Microscopic features. Use of different media induced no marked hyphal variations. The mats, both aerial and submerged, were formed of delicate, colorless, hyaline threads, 1-7 μ wide, and provided with walls of moderate and uniform thickness. The majority of the hyphae were 2-3 μ or 6-7 μ in diameter, intermediate widths being somewhat infrequent. In young cultures all were filled with somewhat vacuolated, granular contents, but with age those of the aerial growth were often empty. The threads were branched and septated, and in the submerged layers spherical swellings, filled with dense contents, were not infrequent (Fig. 96); in the aerial mat clusters of anastomosing threads, and helicoid hyphae (Fig. 94) were of common occurrence. In the layer just below the agar surface, branching was most abundant, resulting in the production of a more or less tenaciously woven layer somewhat difficult to tear.

Clamp connections were noted, but were infrequent when compared with their abundance on other mycelia here described. They

occurred on broad hyphae, and were themselves large and conspicuous. They were found singly, in pairs, or whorls around the septa where they appeared (Fig. 95 *a-h*), and suggested again the characteristic *Coniophora* clamping described by Möller, Rumbold, and Falck, and previously noted in connection with the mycelium of balsam rot Type A (2). Frequently imperfect clamping was noted, the short branch often almost encircling the hypha without effecting fusion (Fig. 95 *a*); and again many were noted where fusion had occurred when no septum appeared in the main hypha (Fig. 95 *b*). No detailed observations of fusion were made, but in many whorls cases were noted in which one or more of the branches had failed to fuse, and others were projecting side branches alternated with clamping hyphae. Sometimes short branches circling the hyphae from opposite sides seemed directed toward fusion with one another. Clamp connections were noted in abundance in actively growing cultures only.

7. Comparison of Growth on Different Media

Experience seems to show that in selecting media with the object of promoting conspicuous differential vegetative peculiarities series of vegetable agars are scarcely to be recommended. In the case of *Polyporus schweinitzii*, as previously recorded, the following were employed: beet, oatmeal, bean, corn, pea, potato, celery, and carrot. Differences occurred, but mainly corresponding with the suitability of the substrata for promoting active growth. The fundamental type of development remained the same if the organism was nourished. Bean, for example, proved unsuitable, and carrot favorable to this fungus; but this did not apply to *Polyporus schweinitzii* alone. *Fomes pinicola* reacted in the same manner, and a comparison with the tables of Long and Harsch (31) for *Fomes texanus, F. rimosus, F. robineae*, and *Polyporus farlowii* show a corresponding response for these organisms. It would seem that scanty growth on bean agar is of little significance for purposes of identification. On celery, on the other hand, *Polyporus schweinitzii* produced, although with less luxuriance, the same filmy spread as was obtained on carrot, while on both media *Fomes texanus* and *Polyporus farlowii* presented respectively their velvety and cottony textures. Such media may serve to impress the constancy of inherent types of growth, but an accumulation of the minor peculiarities exhibited is unnecessary for diagnostic purposes.

Of the media tested for several organisms corn agar proved the least satisfactory. *Fomes applanatus, F. fomentarius, F. pinicola, Polyporus sulphureus, Polystictus versicolor*, and *Trametes pini* each

produced thin, white to colorless films by the end of two weeks. These were not identical, but the peculiarities were insignificant, and further use of the medium was deemed unprofitable. Addition of dextrose provided an essential nutrient, but growth then approached that obtained on potato-dextrose agar. Plain potato agar has proved a very satisfactory medium and, using it as a substratum, a series of cultures of the seventeen organisms under consideration presents at once a basis for diagnosis. For the most part, however, the addition of dextrose provides such an intensification of types that the value of its use is at once recognized. Some few organisms, *Polyporus borealis*, *Poria subacida*, *Fomes igniarius*, *Fomes pinicola* and *Balsam rot Type B* (*2*), find equal or in some cases slightly better initial growth conditions on the simpler medium, but in all cases the additional supply of carbohydrate is in the end found advantageous. *Fomes applanatus*, *F. fomentarius*, *F. igniarius*, *F. pinicola*, and *Polystictus versicolor* were tested on prune and carrot agars; all grew well, but exhibited no striking features not found on potato-dextrose.

It seems reasonable to suppose that greater possibilities of influencing the habit of growth lie in the use of synthetic media, in which by a variation of the source of some essential nutrient physiological changes, resulting in visible alterations in growth, may be induced. Little, however, has been done in this direction. As stated above the addition of a known supply of carbohydrate to a natural vegetable agar was found beneficial. The resulting changes included increased growth, intensification of color in pigmented forms, and in some cases a better differentiation of hyphal types.

Czapek's nutrient solution solidified, having carbon supplied by dextrose and nitrogen by sodium nitrate was tested throughout, and comparison made with a medium in which the nitrate was replaced by peptone. On the whole the first proved an unsuitable substratum. It was decidedly unsatisfactory for *Fomes fomentarius* and *Polystictus abietinus*, afforded but little growth of *Fomes applanatus*, *F. igniarius*, and *Trametes pini*, and while it provided in other cases mats covering the agar slants, these were for the most part thin. It added some interesting features, but none of sufficient moment to warrant its adoption for general comparative work. First the rather marked differences in amount of growth exhibited in the series emphasized the dissimilarity in food requirements. Pigmentation was developed in *Polystictus versicolor* and rendered more uniform in *Fomes igniarius* and *Fomes roseus*; deep staining of the agar was found constant in *Fomes igniarius* and *Polyporus schweinitzii*. Most noticeable of all was the thickening of hyphal walls in the majority of cases, rendering

conspicuous, for example, the delicate, septated hyphae of *Fomes pinicola* and the delicate clamp connections of *Fomes applanatus*. Reaction to a substitution of peptone for nitrate was most decided in all cases. The mats then produced, approached in luxuriance those developed on potato-dextrose and malt, and will be considered in a comparison with those media.

These three media, *potato-dextrose*, *malt*, and *Czapek's synthetic (modified) agars*, each furnished ample nutriment for the development of luxuriant mats of the fungi studied. Potato-dextrose and malt were almost equally good, the one or the other proving in individual cases slightly superior in respect to amount of growth. On the whole potato-dextrose was found preferable, since the mats were more closely woven and, as a consequence, presented surfaces of more definite character. Illustration of resultant peculiarities were the marked rolling of *Fomes applanatus* mats and the distinct furrowing of *Polystictus abietinus* on potato-dextrose, as opposed to the smooth surfaces produced on malt. In cases where fibre-like hyphae did not form the predominant element of the aerial growth, it was noted that their production was more abundant on potato-dextrose. Cases in point are found in the development of *Fomes roseus*, and *Balsam rots Type A* (*2*) and *Type B* (*2*). The difference in hyphal differentiation doubtless features also in the case of the softer growth of *Fomes applanatus*, *Fomes fomentarius*, *Trametes pini*, etc., on malt, but owing to the very profuse development of fibre-like hyphae even on that medium a comparison of the relative numbers is impossible. The rule is not without exception, however, for in the case of the strain of *Fomes igniarius* from poplar fibre-like hyphae developed more frequently in malt cultures. This circumstance and the characteristic deposition of crowns of crystals on the ends of short side branches in the aerial growth of *Polystictus abietinus* were the most valuable diagnostic peculiarities developed on malt. Otherwise growth on potato-dextrose was found to yield mats with characteristics slightly more pronounced.

Growth on *Czapek's synthetic (modified)* agar effected decided changes in the mats produced, but the most noticeable variations were common in a more or less marked degree to all the organisms tested. There occurred a considerable thickening of the mats, accompanied by a noticeable retardation of spread, as compared with growth on potato-dextrose and malt. *Fomes roseus*, *Polyporus balsameus*, *Polystictus versicolor*, and *Balsam rot Type C* (*1*) were least affected, while at the other extreme were *Fomes fomentarius*, *Trametes pini* and *Balsam rot Type A* (*2*). The surface resembled for the most

part the growth on potato-dextrose, but distinct types were developed in some forms, notably in *Balsam rot Type A* (*2*), *Fomes fomentarius*, *F. igniarius* from poplar, and *Trametes pini*. In all cases the mats formed were of softer texture than on potato-dextrose owing to the lack or scanty development of fibre-like hyphae. Very deep and general pigmentation was obtained in *Balsam rot Type A* (*2*) and in *Trametes pini*, and the delicate coloration of *Fomes pinicola* and *F. roseus* was accentuated; in the case of *Fomes applanatus* and *F. fomentarius*, however, the reverse proved true, the cultures on the synthetic medium being less deeply tinted than those on potato-dextrose. Staining of this agar was more frequent than of other media, but the point has not been considered with care. Inoculated with *Polyporus schweinitzii* the agar turned black very rapidly in most cases and initial growth was delayed. Final growth, however, agreed with that in unstained tubes and, with increasing growth, the agar bleached. *Trametes pini* and *Balsam rot Type A* (*2*) caused a gradual staining, which eventually turned black. This proves a good differentiating medium; but the poorer development of fibre-like mycelium marks it as inferior to potato-dextrose and malt. On the other hand, it adds characteristics not obtained upon them.

The organisms studied stand, however, so distinct from one another upon each of the three media, that for purely diagnostic purposes any one of them will provide sufficient morphological distinctions to insure identification. As before stated, potato-dextrose agar seems to offer a slight superiority, and the peculiarities afforded by that medium are summarized below.

8. Identification by Growth on Potato-dextrose Agar

(a) *Key.*

Mats white or faintly yellowed during a month's growth.

Mats radially furrowed. — 10. *Polystictus abietinus.*

Mats not furrowed.

Chlamydospores present. — 7. *Polyporus borealis.*

Chlamydospores absent.

Fibres present.

Fibres sparingly branched.

Fibres uniform. — 12. *Poria subacida.*

Fibres with expansions. — 11. *Polystictus versicolor.*

Fibres much branched. 15. *Balsam rot Type B (2).*

Fibres absent.

Clamp connections always single. 16. *Balsam rot Type B (3).*
Clamp connections usually whorled. 17. *Balsam rot Type C (1).*

Mats faintly pink.

Chlamydospores present. 9. *Polyporus sulphureus.*
Chlamydospores absent.

Hyphae with clamp connections very delicate. 4. *Fomes pinicola.*
Hyphae with clamp connections more heavily walled. 5. *Fomes roseus.*

Mats variously and often deeply colored, not as above.

Chlamydospores present.

Clamp connections present. 6. *Polyporus balsameus.*
Clamp connections absent. 8. *Polyporus schweinitzii.*

Chlamydospores absent.

Clamp connections present.

Clamp connections always single.

Fibres coarse and darkly pigmented. 2. *Fomes fomentarius.*
Fibres delicate, mostly hyaline. 1. *Fomes applanatus.*

Clamp connections usually whorled. 14. *Balsam rot Type A (2).*

Clamp connections absent.

Fibres uniform. 3. *Fomes igniarius.*
Fibres with expansions. 13. *Trametes pini.*

(b) *Summary of diagnostic characteristics.*

1. *Fomes applanatus* (Figs. 1 and 55-58).

Mats skin-like with felty to farinaceous surface, old mats rolled; coloration varied, including: pale ochraceous, pinkish and tileul buff, avellaneous, vinaceous fawn, ecru olive, light cinnamon drab, citrine drab, and greyish olive; spread rapid: slants covered in 12-14 days.

Submerged mycelium: hyphae delicate, hyaline, colorless, thin-walled, branched, septated, 4-10 μ wide, with inconspicuous clamp connections; large swollen cells often massed into compact layers at various depths. *Aerial mycelium:* (1) advancing zone and part of older mat similar to submerged but without spherical expansions; (2) fibre-like threads with thick walls and narrow lumina, 1-4 μ wide, branched, sparingly septated, tightly interwoven, producing witches' brooms in old cultures.

2. *Fomes fomentarius* (Figs. 16 and 63-66).

Mats forming thick, close felts, becoming leathery to crust-like with age, with smooth surface; coloration: pinkish buff, light ochraceous salmon, ochraceous tawny, cinnamon, sayal, and Prout's brown; spread moderate: slants covered in about three weeks.

Submerged mycelium: hyphae delicate, colorless, hyaline, 2-7 μ wide, much branched and septated, numerous clamp connections. *Aerial mycelium:* (1) advancing zone and part of older growth similar to the submerged mycelium; (2) fibre-like hyphae of uniform width, with thick colorless to greenish walls and colorless to dark brown contents, sparingly branched and septated, 1-5 μ wide.

3. *Fomes igniarius* (Figs. 11 and 89-93).

Mats thick, with rough, tufted woolly to fairly smooth, soft, felty-woolly surface; coloration: cartridge, cream and cinnamon buff, chamois, honey yellow, Isabella color, clay color, tawny olive, and cinnamon brown; spread moderate: slants covered in about three weeks.

Submerged mycelium: hyphae delicate, colorless, hyaline, 2-4 μ wide, loosely branched, with frequent septation and no clamp connections; in old cultures many irregular expansions, often compacted into one or more layers, which become darkly pigmented. *Aerial mycelium:* (1) advancing zone and part of older growth similar to the submerged, but without expansions; (2) fibre-like hyphae coarse, 2-4 μ wide, uniform, sparingly branched, infrequently septated, with smooth, yellowish green walls and yellowish to dark brown contents.

4. *Fomes pinicola* (Fig. 3).

Mats delicate, easily torn, loose, dry, downy, woven later to a soft felt with smooth, felty-woolly to matted woolly surface; coloration: white, shadowed with pale ochraceous buff to pale ochraceous salmon; spread rapid: slants covered in 12-14 days.

Submerged mycelium: hyphae delicate, colorless, hyaline, 2-6 μ wide, branched, septated, with numerous clamp connections, walls thin and contents coarsely granular. *Aerial mycelium:* (1) advancing zone and part of older mat similar to the submerged, but especially in the latter less vigorous in appearance, 1-2 μ or occasionally 4 μ wide; (2) fibre-like hyphae abundant and formed in young mats, hyaline, colorless, 1-4 μ wide, thick walled, uniform, sparingly branched, unseptated.

5. *Fomes roseus* (Figs. 10 and 46).

Mats light, flocculent-downy, later becoming closely woven, with even felty-woolly surface; coloration: white, becoming in three weeks shadowed with light ochraceous salmon, and later intensified to light vinaceous cinnamon and vinaceous buff with staining of avellaneous; spread moderate: slants covered in about three weeks.

Submerged mycelium: hyphae delicate, hyaline, colorless, 1-3 μ wide, septated, branched, with numerous clamp connections; in old mats often very irregular in outline and septation, averaging 6 μ in width; a few deeply pigmented. *Aerial mycelium:* (1) advancing zone similar to the submerged; (2) older growth partly similar to the submerged, many becoming deeply pigmented, showing frequent anastomosis; (3) fibre-like hyphae not developed during the first ten days but when formed colorless, hyaline, 2-3 μ wide, with thick, refractive walls, sparingly branched and septated.

6. *Polyporus balsameus* (Figs. 26 and 73-76).

Mats at first thin, downy-hairy, with broad, straggling, colorless, appressed advancing zone, becoming close woolly to light downy with farinaceous area in the vicinity of the inoculum, and finally assuming a farinaceous to spongy texture over the whole surface; coloration: white, gradually deepening through cream buff and cinnamon buff to an even drab, with occasional honey yellow patches; spread moderate: slants covered during the third week.

Submerged mycelium: (1) hyphae delicate, 2-6 μ wide, colorless, with firm, hyaline walls, branched and septated, producing in old

cultures large, conspicuous, irregular expansions singly or in series, clamp connections numerous; (2) chlamydospores, developed during the first week, usually in fine hyphae about 2 μ wide, elliptical to subspherical and 10 x 12 μ in size, or irregular and ranging 7-10 x 12-22 μ, terminal or intercalary, with thick, smooth, hyaline walls and colorless to yellowish contents. *Aerial mycelium:* (1) advancing zone and part of older mat similar to the submerged; (2) chlamydospores often produced terminally in spray-like clusters, but also in intercalary position forming chains, contents yellowish to deep brown; (3) coarse hyphae similar in type to the above, but with thicker walls and frequent sac-like expansions, all filled with dense, drak brown contents.

7. *Polyporus borealis* (Figs. 50 and 77-80).

Mat thick and soft, soon tending to fill the base of the tube, with felty to felty-velvety surface, becoming thinner toward the top and developing there a farinaceous surface; felty balls and ridges over surface; sporophores not infrequent; coloration: white or faintly cream color; spread slow: slants covered in five weeks, but mats thick.

Submerged mycelium: (1) hyphae fine, regular, 2-6 μ wide, much branched and septated, clamp connections abundant, walls firm, colorless, hyaline, refractive; (2) chlamydospores abundant, spherical, subspherical, elliptical, pear shaped, or irregular, with smooth, hyaline walls, and coarsely granular contents, 6-10 x 10-20 μ in size. *Aerial mycelium:* (1) advancing zone and part of older mat similar to submerged; (2) chlamydospores as above; (3) fibre-like hyphae developed in old cultures, with thick walls and narrow lumina, sparingly branched and septated, often irregular in outline, clamp connections infrequent.

8. *Polyporus schweinitzii* (Figs. 21, 23 and 67-72).

Mats thin, light, furry-filmy with irregular thickened balls and stalks with velvety surface, when exposed to light more compact, smooth and with velvety surface; coloration: barium yellow with thickened areas honey yellow to buckthorn brown, when exposed to light vivid—sulphine, strontian, citron and barium yellow with brownish shades of honey yellow, raw sienna, buckthorn brown, etc.; spread rapid: slants covered in less than two weeks; mats exposed to light: slants covered in about three weeks.

Submerged mycelium: (1) hyphae delicate, hyaline, frequently branched and septated, no clamp connections, 3-5 μ wide; (2) chlamy-

dospores abundant, smooth walled, spherical, subspherical or elliptical, terminal or intercalary, 6-16 x 8-20 μ in size, pigmented as the hyphae in which they are formed. *Aerial mycelium:* (1) advancing zone and part of the older mat similar to the submerged; (2) chlamydospores as above; (3) very regular hyphae 3-5 μ wide, with smooth, colorless to greenish walls of moderate and uniform thickness, and yellowish to reddish brown contents, septated and branched, branches often bent abruptly at point of origin and lying appressed to parent hypha, without spores except in old mats, formed into a thick velvet pile in mats exposed to light.

9. *Polyporus sulphureus* (Figs. 2 and 99-106).

Mats soft, close, light and downy, becoming somewhat spongy with age; coloration: white, deepening through pale ochraceous buff and pale ochraceous salmon to light ochraceous salmon; spread moderate: slants covered during the third week's growth.

Submerged mycelium: (1) hyphae rather delicate, hyaline, loosely branched, frequently septated, often constricted at the septa, with no clamp connections, variable in width, 3-12 μ and in old cultures up to 22 μ in diameter; (2) chlamydospores irregular in outline, terminal or intercalary, 10-13 x 18-30 μ in size, with relatively thin walls. *Aerial mycelium:* (1) advancing zone and most of the older growth of hyphae similar to those in the submerged layers; (2) chlamydospores borne terminally in spray-like clusters or forming in series in intercalary position, the former subspherical, the latter varied in outline, 6-7 x 6-11 μ in size; (3) fine, fibre-like hyphae found in old cultures, 1-3 μ wide, with thick walls, narrow lumina, infrequent branching, and septation.

10. *Polystictus abietinus* (Figs. 41 and 87).

Mats at first thin with farinaceous surface, later closely woven, skin-like, with felty-woolly surface and deep radiating furrows; coloration: white, becoming sodden; spread moderate: slants covered during the third week.

Submerged mycelium: hyphae hyaline, colorless, firm-walled, branched, septated, 2-4 μ wide, with an abundance of conspicuous clamp connections. *Aerial mycelium:* (1) advancing zone similar to the submerged; (2) older growth similar to the submerged but with thicker walls, and with a tendency to form short upright branches with many irregular enlargements, hyphae 3-5 μ wide.

11. *Polystictus versicolor* (Figs. 42, 81 and 83-86).

Mats closely woven, new growth downy, later felty, finally becoming smooth and chalky; coloration: white, becoming yellowed and sometimes showing patches of old gold; spread rapid: slants covered in 12 days.

Submerged mycelium: hyphae colorless, hyaline, much branched and septated, with numerous clamp connections, 2-6 μ wide, in old mats often very irregular in outline and greatly enlarged. *Aerial mycelium:* (1) hyphae similar to those of the submerged; (2) colorless, fibre-like hyphae, 2-5 μ wide, with thick, hyaline walls and narrow lumina, infrequent branching and septation, very infrequent clamp connections, and frequent terminal and intercalary expansions with very thick walls.

12. *Poria subacida* (Fig. 44).

Mats at first light, flocculent-downy, with broad, even, appressed advancing zone, becoming close downy and finally matted downy to matted woolly; coloration: white, becoming gradually yellowed, discoloration spreading from the top irregularly over the surface; spread rapid: slants covered in two weeks.

Submerged mycelium: hyphae delicate, colorless, 2-6 μ wide, with firm, hyaline walls and coarse granular contents, clamp connections numerous. *Aerial mycelium:* (1) advancing zone of vigorous, turgid hyphae similar to the submerged; (2) part of older growth similar to submerged; (3) fine, fibre-like hyphae, 1-3 μ wide, sparingly branched and septated, with thick, hyaline walls and narrow lumina, furnished at first with granular contents but soon becoming empty.

13. *Trametes pini* (Figs. 31 and 59, 60 and 62).

Mats thick and soft, with rough, tufted woolly surface; coloration rich including: amber, mustard, aniline and honey yellow, old gold, orange citrine, clay color, tawny olive, and buckthorn brown; spread slow: slants covered during the fourth week, but mats thick.

Submerged mycelium: (1) hyphae delicate, 1-3 μ wide, regular, branched, septated, with thin, colorless, hyaline walls; (2) hyphae 1.5-4 μ wide, firm, regular, with relatively thick, hyaline, colorless or slightly greenish walls, and contents colorless to greenish or yellowish brown, branched, septated, with septa often arched, presenting numerous enlargements along their course, closely interwoven in the superficial agar layer. *Aerial mycelium:* (1) advancing zone similar to the first type of the submerged; (2) older growth showing both of

the submerged types; (3) coarse, fibre-like hyphae, 1.5-5 μ wide, with thick colorless to greenish walls and yellowish to dark brown contents, branched and septated but less frequently than other hyphae, with numerous expansions often occurring in series, formed from the second type of much-branched hyphae and often reproducing them again after considerable elongation.

14. *Balsam rot Type A* (*2*) (Figs. 4, 5 and 97).

Mats at first semi-appressed, silky, with more or less prominent radiating strands, later becoming more closely woven with felty-woolly surface, and finally producing toward the base a more or less warted felt and toward the top a skin- or crust-like growth covered with a matted tangle of long, loose hyphae; coloration: white, soon becoming massicot yellow, in old cultures growth at the base white and baryta yellow and toward the top almost buckthorn brown covered with a light, matted, pale yellow tangle; spread rapid: slants covered in 10-12 days.

Submerged mycelium: (1) delicate, colorless, hyaline hyphae, 2-8 μ wide, thin-walled, sparingly septated, branched usually at right angles; (2) broad, irregular, thin-walled hyphae, 10-12 μ wide, usually with colorless but at times yellowish brown contents, branched, septated, often constricted at the septa, clamp connections conspicuous, single, in pairs, or whorls around the septa but infrequent in old cultures. *Aerial mycelium:* (1) hyphae similar to those of the submerged mycelium; (2) fine, hyaline, fibre-like threads, 1-2 μ wide, with thick walls and narrow lumina, unseptated, branched often at right angles. In old cultures fine hyphae of the first formed type, 0.5-3 μ in width, supply the most abundant element.

15. *Balsam rot Type B* (*2*) (Fig. 4).

Mats at first thick, soft and silky, soon tightening down and matting, but with advancing growth remaining soft and silky, becoming gradually a downy to downy-woolly tangle, finally producing a dry, thin, smooth, soft mat of delicate texture; coloration: white, dotted by the end of a week with bands or irregular patches of yellow moisture drops, mycelium staining pinkish, warm, or light buff in the vicinity of the excretion but these patches gradually overgrown, and in old mats discoloration of cream buff spreading from upper edge irregularly over the surface; spread rapid: slants covered in two weeks.

Submerged mycelium: hyphae strong, colorless, 2-6 μ wide, with firm, hyaline walls, and numerous clamp connections, much branched

and septated, and often displaying great irregularity of outline. *Aerial mycelium:* (1) advancing zone of delicate, regular, turgid hyphae similar to those of the submerged; (2) most of the older growth formed of threads similar to those of the submerged, colorless except in the yellowed areas, where the contents were faintly to decidedly yellow; (3) fine, colorless fibre-like hyphae, 1-2 μ wide, developed toward the end of a month's growth, with comparatively thick walls and narrow lumina, unseptated and profusely branched.

16. *Balsam rot Type B (3)* (Fig. 49).

Mats thin, farinaceous-filmy to matted hairy with rather regular, colorless, appressed advancing zone, becoming closely woven, smooth, felty-woolly to rough, hairy-woolly surface; coloration: white, becoming even ivory yellow: spread slow: slants covered in three to four weeks.

Submerged mycelium: hyphae delicate, colorless, 1-3 μ (up to 7 μ in young cultures) wide, with uniform, hyaline walls and numerous clamp connections, branched, septated, very closely interwoven just beneath the agar surface, then passing to a looser tangle in the deeper layers, many becoming empty in old cultures. *Aerial mycelium:* (1) advancing zone of hyphae similar to the submerged, but turgid, 2-5 μ wide, and filled with fine, granular contents; (2) older growth of hyphae similar to the submerged, 0.5-3 (mostly 2 μ) wide, for the most part devoid of contents and with clearly defined walls of moderate thickness.

17. *Balsam rot Type C (1)* (Figs. 43 and 94-96).

Mats soft, shaggy downy, with colorless, semi-appressed, regular advancing zone, becoming thick, soft and downy-woolly, dotted with glistening, colorless moisture drops, finally somewhat felted with matted surface and in old cultures forming closely felted, circular ridges or shelves in the vicinity of the inoculum; coloration: white, becoming faintly cream color in the vicinity of the inoculum and in some cases developing patches of baryta yellow; spread rapid: slants covered in 10-12 days.

Submerged mycelium: hyphae delicate, colorless, 1-7 μ wide, the majority 2-3 or 6-7 μ, intermediate widths being rare, with hyaline walls of moderate and uniform thickness, branched and septated, presenting spherical swellings with dense contents, thickly matted just below the agar surface to form a tenacious layer, conspicuous but not abundant clamp connections found singly, in pairs, or whorls on

the broad hyphae. *Aerial mycelium:* similar to the submerged, hyphae showing frequent anastomosis and helicoid formation, the majority becoming with age devoid of contents.

9. Influence of Host upon Cultural Characteristics

Table I

Fungus	No. of sources	No. of hosts
Fomes applanatus	8	5
" *fomentarius*	5	2
" *igniarius*	25	8
" *pinicola*	19	8
" *roseus*	1	1
Polyporus borealis	5	2+*
" *schweinitzii*	6	1+*
" *sulphureus*	4	1+*
Polystictus abietinus	8	2
" *versicolor*	4	3
Trametes pini	6	3+*
Balsam rot Type A (1)	6	2
" " " " (2)	17	2
" " " *B* (1)	10	2
" " " " (2)	11	2
" " " " (3)	6	1
" " " *C* (1)	6	1

Of the thirteen known organisms studied *Fomes igniarius* alone, as mentioned above, presented variations deemed traceable to host influence; but the material at hand, as indicated in Table I, leaves much to be desired in the matter of possible host range for the several fungi considered; and the presence of one recognizable strain among the cultures observed points to the desirability of including mycelium from all possible hosts, before deciding on the constancy of the vegetative characteristics.

10. Secondary Spore Forms

Among organisms of which the majority of the mycelia, under the environmental conditions provided, have proved sterile the occurrence of secondary spore forms offers at once a striking diagnostic characteristic. Of the seventeen fungi considered in this study, four, all belonging to the genus *Polyporus* and indeed the only members of

*Hosts not recorded for all sources.

that genus investigated, produced on all media upon which satisfactory vegetative growth was obtained an abundance of chlamydospores. A fifth fungus, *Polystictus versicolor*, was prolific in the production of chlamydospores on Czapek's synthetic (modified) agar only, among the media tested. It, therefore, suggests that among mycelia here found sterile, sporulation may be attained by variation of growth conditions, and supports the possibility of adding important diagnostic criteria by a choice of suitable differential media. Chlamydospore formation in all five agreed with that described by Lyman (32) in his treatment of secondary spores among the Hymenomycetes.

The extensive history by Lyman (32) upon observations of the occurrence of secondary methods of reproduction among the Basidiomycetes obviates the necessity of a further review; and a more recent paper by Snell (49) adds many references upon subsequent investigations. Lyman reported secondary spore forms in about 40% of the seventy-five species of Hymenomycetes which he investigated, and found chlamydospores in over one quarter of the species cultivated, a much more common occurrence than was previously known. Of the five timber-destroying fungi considered by Snell two produced both oidia and chlamydospores, two others chlamydospores, while the fifth remained sterile.

Polyporus sulphureus. The occurrence of secondary spores, termed conidia, was reported by de Seynes (48) in connection with natural basidial fructifications of *Polyporus sulphureus* in 1878. They were not found in all sporophores examined, but their development seemed in inverse proportion to the production of tubes and, as a result, to basidiospore formation. If the development of primary fructifications were made an object of cultural studies the conditions here employed would scarcely find favor, since the abundant formation of secondary spores stands opposed to the probability of their production. The conidia of de Seynes correspond to the chlamydospores here noted. They are distinguished from those of *Polyporus balsameus*, with which of the forms here discussed they agree most closely, by their greater variability in size, more uniform granular contents, and delicacy of appearance. In both of these fungi practically the entire aerial mat in old cultures formed a pulverulent mass of spores. Among those produced terminally in *Polyporus sulphureus* examples 5 x 6 μ were not infrequent, while in the same mount spores up to 13 x 30 μ might appear. The largest spores came from the submerged growth, but intermediate forms were provided by the intercalary cells

of the aerial mat. All were colorless and provided with smooth, hyaline walls.

Polyporus balsameus. The original description of *Polyporus balsameus* by Peck (37) in 1878 contained no reference to secondary spore forms, nor indeed have subsequent observations added any information concerning their occurrence in nature. The present report appears to be the first record of such spores in connection with this organism. Among the fungi here treated these spores are easily recognized as characteristic in form. Those developing terminally are rather uniform in size and shape: 8-10 x 10-12 μ, and on the whole subspherical. In intercalary position irregular spores were often developed, but in the powdery mass found in old mats, these formed an inconspicuous feature. In all, the walls were smooth and fairly thick, and the contents, varying from greenish to yellowish brown, assumed for the most part a conspicuous concentric arrangement.

Polyporus borealis and *P. schweinitzii.* No previous records of secondary spores in either of these fungi are known to the writer. Descriptions of both organisms date back to Fries' earliest classification of 1821. Careful anatomical studies of sporophores were also included by Hartig (25) in his investigations of wood-destroying fungi. No appearance of secondary spores, occurring either naturally or under artificial conditions has, however, been noted. Differentiation between these two organisms by spore characteristics would be impossible. In both the spores are subspherical, elliptical to irregular in shape, and very variable in size. The walls are of moderate thickness, smooth, colorless and hyaline, and the contents coarsely granular. Pigmentation may occur in *Polyporus schweinitzii*, but colorless spores are more numerous.

Polystictus versicolor. As noted above secondary spore production is varied and of common occurrence in *Polystictus versicolor.* Conidia occurring naturally were reported by Patouillard (36) and in culture by Bayliss (2); oidia produced under artificial conditions were noted by Brefeld (7), Bayliss (2) and Lyman (32). No former record of chlamydospore formation has been found. In the cultures under observation this was the only type of spore developed. They were formed abundantly in the mycelium produced on Czapek's synthetic (modified) agar. In character they did not differ materially from the sporesof *Polyporus borealis* or *P. schweinitzii.* The maximum size recorded was found to be somewhat less than in the other species, but great variability in size and shape prevailed.

11. Sporophore Production

Until the publication of the work of Long and Harsch (31) in 1918 no method of obtaining fructification of fungi belonging to the Polyporaceae on artificial media had been introduced. The cultural studies of the Basidiomycetes by Brefeld formed the starting point for extensive and varied investigations, the scope of which is ever broadening. His aim, however, the study of the complete life history of an organism under controlled conditions, has even yet been reached in comparatively few cases among the higher fungi. His own results covered several of the Agaricaceae including species of Coprinus, Paneolus, Stropharia, Psathyra, Galera, Marasmius, and Nyctalis (7). The first wood-destroyer brought to maturity was *Collybia velutipes*, cultured by Costantin and Matruchot (15). The researches of Ward (52), Biffin (3), Falck (18 and 19), Spaulding (50), Buller (9), Price (39), Bayliss (2), White (55), and Hiley (30) offer other examples of successful fructification of wood-destroying forms from the Thelephoraceae, Polyporaceae, and Agariceae, but among these Falck's work on the growth of Merulius, *Polyporus betulinus*, etc. (19), and his studies of Lenzites (18) alone record mature sporophores of members of the Polyporaceae upon artificial media. The signal success in this respect which resulted from the experimental treatment employed by Long and Harsch (31) has been already mentioned. Among the Polyporaceae they report sporophore production in species of Fomes, Polyporus, Ganoderma, Polystictus, Trametes, Daedalea, Lenzites, Merulius and Poria. Of the species included in the present investigation they were successful in the culture of *Fomes applanatus*, *F. pinicola*, *F. roseus*, *Polyporus sulphureus*, and *Polystictus versicolor*. While the fertility of the sporophores obtained is mentioned in general terms no definite information as to sporulation in individual organisms is recorded, nor are descriptions of the sporophores produced offered.

In the present investigation fruit body production has been considered quite incidentally, no effort having been made to vary the growth conditions with a view to favoring fructification. It is probable that the elimination of light may account to a considerable extent for the sterility of most of the cultures, which were grown under conditions otherwise quite similar to those used by Long and Harsch. They subjected their cultures to daylight and ordinary room temperatures; the organisms here considered were grown in practical darkness and at constant temperature. The latter is to be preferred for uniformity of mycelial growth, but the former would doubtless be found more favorable to fructification. Light was found

to be a decidedly important factor, 629 sporophores having been obtained by Long and Harsch in the light as compared with 11 in darkness. These results agree with former observations. Brefeld (7) noted the sterility of certain Coprini in darkness, and the imperfection of fructification of other forms of the same genus when deprived of light. These organisms all fruited normally when illuminated, and indeed after the initiation of fructification were able to mature their sporophores in darkness. Buller (8) in an investigation into the reactions of fruit bodies of *Lentinus lepideus* to external stimuli found that the papillae from which fructifications developed were formed equally well in light or darkness; but in the absence of light excessive elongation of the stipe without the formation of a pileus resulted, while illuminated cultures presented normal fructifications. Again in *Polyporus squamosus* (11) he obtained only elongated, aborted, and sterile fruit bodies in darkness as opposed to normal development in light; but he noted that temporary exposure to daylight, even for a single hour, resulted in the subsequent development of pilei from the rudimentary structure obtained in darkness.

Such fructifications or indications of fructification as were obtained in the course of the present study were produced in comparative darkness, since they developed in cultures placed in thermostats, or in stock series stored in cardboard boxes. All, however, were exposed occasionally to light, when the tubes were removed for examination. The sporulating culture of *Polyporus borealis* shown in Fig. 45 was not subjected to standardized conditions, but developed in stock series. Other fruit bodies of the same organism, however, developed and sporulated, producing viable spores, in tubes incubated at 22° C. Indeed fructification in this case seemed favored by darkness. Four erlenmeyer flasks containing upright potato-dextrose agar surfaces were inoculated with this fungus and placed on a shelf illuminated by diffuse sunlight. Two were carefully wrapped in black paper. In one of those from which light was excluded sporulating fruiting surfaces developed; the others remained sterile. It would seem that light is in this case inessential, although its exclusion does not guarantee complete development. That the absence of light does not inhibit fructification in all the higher fungi is evidenced by the observations of Spaulding (51) on fruit bodies occurring in clay mines. While all the organisms noted by him were not found in full maturity, many species including *Merulius rubellus*, *Fomes annosus*, *F. applanatus*, *Lenzites betulina*, *Polystictus versicolor*, and others were found fruiting normally.

Other than the fully matured sporophores of *Polyporus borealis* already recorded, the only suggestions of fructification noted were found in the daedaloid surfaces produced in several subcultures by one strain of *Fomes pinicola*, the balls and stalks developed in *Polyporus schweinitzii* cultures, and the ridges and bracket-like outgrowths formed by *Balsam rot Type C (1)*.

12. Summary

1. Experience has shown that means should be established for testing and supplementing field methods used in the identification of organisms responsible for the heartwood and sapwood rots which are so prevalent and destructive in our forests. With this end in view an attempt has been made to elaborate a system of laboratory cultural diagnosis. Such a system has been found workable. It may be pointed out that the results confirm in many cases the conclusions drawn from field observations; but in other instances, such for example as in the case of butt rot in balsam commonly ascribed to *Polyporus schweinitzii*, the cultural method has shown that organisms, other than those at present held responsible, may be active in the production of well-known types of decay. The major results of the investigation in this connection are summarized in section 8 of this paper. A tabulated report is there made of investigations on seventeen wood-destroying fungi grown on artificial media. It indicates that these organisms exhibit individual mycelial characteristics sufficiently marked in cultures to make possible the ready recognition of each.

2. Standard cultures were obtained from sporophore tissue. For the majority of the organisms studied such standards were obtained at the outset, and isolations from wood infected with these fungi were found to yield similar mats. Six organisms, however, were first isolated from decayed wood of *Abies balsamea*. Of these, two, owing to their similarity to sporophore tissue cultures, were subsequently identified as the mycelia of *Poria subacida* and *Polyporus balsameus*; two others showed peculiarities suggesting mycelium of Coniophora, as described by Möller (35), Falck (18), and Rumbold (42) (see footnote on p. 261); the mycelia of the remaining two presented distinct types of growth, but nothing suggestive of their identity has appeared.

3. Cultures compared were started from actively growing mycelium, and were subjected to standardized conditions as regards nutrition, light, and temperature. For exact comparative work such standardization is essential, although the majority of organisms were

found to permit of somewhat wide variation in environmental conditions without marked changes in their habit of growth. The decided sensitivity of *Polyporus schweinitzii* to the action of light, however, showed the danger of confusion, if fixed standards were not adopted.

4. For several forms temperature and light conditions were varied, and the effect of changes in acidity of the medium tested. Each set of conditions tried was found to yield variations sufficiently marked and constant to characterize any organism. The range of acid tolerance of the fungi observed was found so great, that exact standardization was deemed unnecessary; but to insure a moderate uniformity, strict rules were observed in the preparation of media. Cultures of different fungi grown in the darkness provided by a non-illuminated thermostat were found to present mats of typical color and texture; and, since incubation at 22° C. permitted moderate and healthy growth, it was adopted for comparative work.

5. Characteristics found of diagnostic value are macroscopic and microscopic. The former deal with habit of growth as to texture, surface, coloration, rate and manner of spread; the latter include hyphal variations with reference to width, regularity of outline, branching, septation, thickness of walls, color, and the occurrence and type of clamp connections and secondary spore forms.

6. Good growth of all forms tested was obtained in suitable liquid media, but for the work in hand a solid substratum was found preferable.

7. The fungi studied were cultured from as great a number of sources as was available. In most cases but a slight range of variation, quite insufficient to effect confusion of forms, was noted in the characteristics displayed by the different organisms. One distinct strain of *Fomes igniarius* was, however, obtained. It was produced by inocula taken from sporophores from different poplar trees, and proved constant for all isolations made from that host.

8. Growth of some forms was observed on several vegetable agars, but for the production of marked, differential characteristics such series were found of little value. The fungi were compared throughout on *potato-dextrose, potato, malt, Czapek's synthetic* and *Czapek's synthetic (modified)* agars. Each medium was found to supply criteria for identification, but most satisfactory development was obtained on potato-dextrose, malt, and Czapek's synthetic (modified) agars. Of these three the first was slightly superior in differentiation of forms, and growth upon it has been made a basis for the separation of organisms.

9. Chlamydospores were found in *Polyporus sulphureus*, and are reported for the first time in *Polyporus balsameus*, *P. borealis*, *P. schweinitzii*, and *Polystictus versicolor*. In the first four organisms they developed on all media, which permitted active growth; but in the fifth they appeared in abundance on Czapek's synthetic (modified) agar only.

10. The production of sporophores was not made a special object of study, but fructification was attained in *Polyporus borealis*. Ten sporophores were produced, and the spores found viable, and capable of reproducing the mats previously found typical of the organism. The fruit bodies developed on potato-dextrose, potato, and malt, and were obtained both in sets grown under standardized conditions and in stock series.

The research was carried on at the University of Toronto during the tenure of a studentship and fellowship awarded by the Honorary Advisory Council for Scientific and Industrial Research of Canada. I am indebted also to the University of Toronto for grants which have made possible the continuance of the work during the summer months.

The problem was undertaken at the suggestion of Dr. J. H. Faull, and to him I wish to express my thanks for the major part of the material from which cultures have been obtained, for photographs of rotted wood reproduced in Figs. 51-54, and in particular for his continued interest throughout the course of the investigation. My thanks are due also to Mr. W. N. Millar, Dr. H. P. Bell, Mr. A. W. Macallum, and Mr. O. Schierbeck for material which they have collected.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. *Fomes applanatus:* culture on potato-dextrose agar, 11 days' growth at 22° C.
Fig. 2. *Polyporus sulphureus:* culture on potato-dextrose agar, 11 days' growth at 22° C.
Fig. 3. *Fomes pinicola:* culture on potato-dextrose agar, 11 days' growth at 22° C.
Fig. 4. *Balsam rot Type A (2):* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 5. *Balsam rot Type A (2):* culture on potato-dextrose agar, 4 weeks' growth at 22° C.
Fig. 6. *Fomes applanatus:* culture on Currie's synthetic (modified) agar, 11 days' growth at 22° C.
Fig. 7. *Polyporus sulphureus:* culture on Currie's synthetic (modified) agar, 11 days' growth at 22° C.
Fig. 8. *Fomes pinicola:* culture on Currie's synthetic (modified) agar, 11 days' growth at 22° C.
Fig. 9. *Balsam rot Type B (2):* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 10. *Fomes roseus:* culture on potato-dextrose agar, 4 weeks' growth at 22° C.

PLATE II

Fomes igniarius: 2 weeks' growth on potato-dextrose agar

Fig. 11. Incubated at 22° C.
Fig. 12. " " 27° C.
Fig. 13. " " 35° C.
Fig. 14. Grown in diffuse sunlight.
Fig. 15. " " position receiving 3 hours' direct sunlight.

Fomes fomentarius: 2 weeks' growth on potato-dextrose agar.

Fig. 16. Incubated at 22° C.
Fig. 17. " " 27° C.
Fig. 18. " " 35° C.
Fig. 19. Grown in diffuse sunlight.
Fig. 20. " " position receiving 3 hours' direct sunlight.

PLATE III

Polyporus schweinitzii

Fig. 21. Culture on potato-dextrose agar, 12 days, room temperature, diffuse sunlight.
Fig. 22. Culture on malt agar: as in Fig. 21.
Fig. 23. Culture on potato-dextrose agar, 12 days, room temperature, darkness.
Fig. 24. Culture on malt agar: as in Fig. 23.
Fig. 25. Culture on potato-dextrose agar, 6 weeks' growth in darkness.

Polyporus balsameus: cultures incubated 18 days at 22° C.

Fig. 26. On potato-dextrose agar.
Fig. 27. On potato agar.

Fig. 28. On malt agar.
Fig. 29. On Czapek's synthetic agar.
Fig. 30. On Czapek's synthetic (modified) agar.

PLATE IV

Trametes pini: cultures incubated 11 days at 22° C.

Fig. 31. On potato-dextrose agar.
Fig. 32. On potato agar.
Fig. 33. On corn agar.
Fig. 34. On Czapek's synthetic agar.
Fig. 35. On Currie's synthetic (modified) agar.

Fomes igniarius: cultures on potato-dextrose agar, 2 weeks' growth at 22° C.

Fig. 36. Culture from sporophore from *Ulmus americana.*
Fig. 37. " " " " *Betula lutea.*
Fig. 38. " " " " *Acer saccharum.*
Fig. 39. " " " " *Betula alba.*
Fig. 40. " " " " *Populus tremuloides.*

PLATE V

Fig. 41. *Polystictus abietinus:* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 42. *Polystictus versicolor:* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 43. *Balsam rot Type C (1):* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 44. *Poria subacida:* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 45. *Polyporus borealis:* culture 2 months' old on potato-dextrose agar, showing fruit body and spore print.
Fig. 46. *Fomes roseus:* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 47. *Fomes roseus:* culture on potato-dextrose agar, 2 weeks' growth at 27° C.
Fig. 48. *Fomes roseus:* culture on potato-dextrose agar, 2 weeks' growth at 35° C.
Fig. 49. *Balsam rot Type B (3):* culture on potato-dextrose agar, 2 weeks' growth at 22° C.
Fig. 50. *Polyporus borealis:* culture on potato-dextrose agar, 2 weeks' growth at 22° C.

PLATE VI

Fig. 51. Longitudinal face of *Abies balsamea* showing rot of Type B.
Fig. 52. Transverse face of *Abies balsamea* showing rot of Type B.

PLATE VII

Fig. 53. Transverse face of *Abies balsamea* showing rot of Type C.
Fig. 54. Longitudinal face of *Abies balsamea* showing rot of Type A.

PLATE VIII

(Figs. 55-106: magnification 810×)

Fomes applanatus

Fig. 55. Hypha with irregular expansions from submerged mycelium on potato-dextrose agar.
Fig. 56. Incrustations of crystals on submerged hypha from potato-dextrose agar culture.
Fig. 57. Fibre-like hypha from aerial mat on potato-dextrose agar.
Fig. 58 (*a-b*). Hyphae of first formed type from potato-dextrose culture.

Trametes pini

Fig. 59. Hyphae from aerial mat on potato-dextrose agar: (*a*) thick-walled hypha with expansions; (*b*) fibre-like hypha; (*c*) showing differentiation of hyphae from first formed type; (*d*) fibre-like hypha with expansions.
Fig. 60. Thick-walled hypha from superficial submerged layer.
Fig. 61. Hemispherical sac-like swellings on aerial hyphae from Czapek's synthetic (modified) agar.
Fig. 62. Hypha of first formed type from growth on potato-dextrose agar.

Fomes fomentarius

Fig. 63. Hypha of first formed type from growth on potato-dextrose agar.
Fig. 64. Thick-walled hypha from submerged mycelium on potato-dextrose agar.
Fig. 65. Thick-walled hyphae from aerial growth on Czapek's synthetic agar.
Fig. 66. Fibre-like hypha from aerial growth on potato-dextrose agar.

PLATE IX

Polyporus schweinitzii

Fig. 67. Hyphae massed in tuft from surface of potato-dextrose agar culture exposed to light.
Fig. 68. Hyphae from surface growth of potato-dextrose agar culture from dark, showing irregular spreading growth.
Figs. 69-70. Hyphae from submerged mycelium of potato-dextrose agar culture.
Fig. 71. Hypha from potato-dextrose agar culture showing developing chlamydospores.
Fig. 72. Chlamydospores from culture on potato-dextrose agar.

Polyporus balsameus

Figs. 73 (*a-e*) and 74. Chlamydospores from potato-dextrose agar culture.
Fig. 75. Hyphae from potato-dextrose agar culture showing irregular expansions: (*a*) aerial; (*b*) submerged.
Fig. 76 (*a-b*). Hyphae showing clamp connections from mat on potato-dextrose agar.

PLATE X

Polyporus borealis

Fig. 77 (*a-d*). Germination of spores.
Fig. 78. Chlamydospores from mat on potato-dextrose agar.
Fig. 79. Fibre-like hypha from aerial mat on potato-dextrose agar.
Fig. 80. Basidium and cystidium from hymenium of fruit body developed on malt agar.

Polystictus versicolor

Fig. 81. Hypha of first formed type from culture on potato-dextrose agar.
Fig. 82. Chlamydospores from mat on Czapek's synthetic (modified) agar.
Fig. 83. Broad irregular hypha from potato-dextrose agar mat.
Fig. 84. Fibre-like hypha from potato-dextrose agar mat.
Fig. 85. Fibre-like hypha showing clamp connections.
Fig. 86. Expansions on fibre-like hyphae.

Polystictus abietinus

Fig. 87. Hyphae from aerial mat on potato-dextrose agar.
Fig. 88. Accumulations of crystals from mats on malt agar.

PLATE XI

Fomes igniarius

Fig. 89. Section through mat on potato-dextrose agar showing compact submerged layer of swollen cells.
Fig. 90. Hypha of first formed type from potato-dextrose agar.
Fig. 91. Hyphae from upper submerged layer on potato-dextrose agar.
Fig. 92. Irregular swollen cells from compact submerged layer on potato-dextrose agar.
Fig. 93. Fibre-like hypha from aerial growth on potato-dextrose agar.

Balsam rot Type C (1)

Fig. 94. Helicoid hyphae from aerial growth on potato-dextrose agar.
Fig. 95 (*a-h*). Variations in formation and arrangement of clamp connections from mat on potato-dextrose agar.
Fig. 96. Enlargements on submerged hyphae from potato-dextrose agar culture.

Balsam rot Type A (2)

Fig. 97 (*a-e*). Variations in arrangement of clamp connections from aerial growth on potato-dextrose agar.

Polyporus schweinitzii

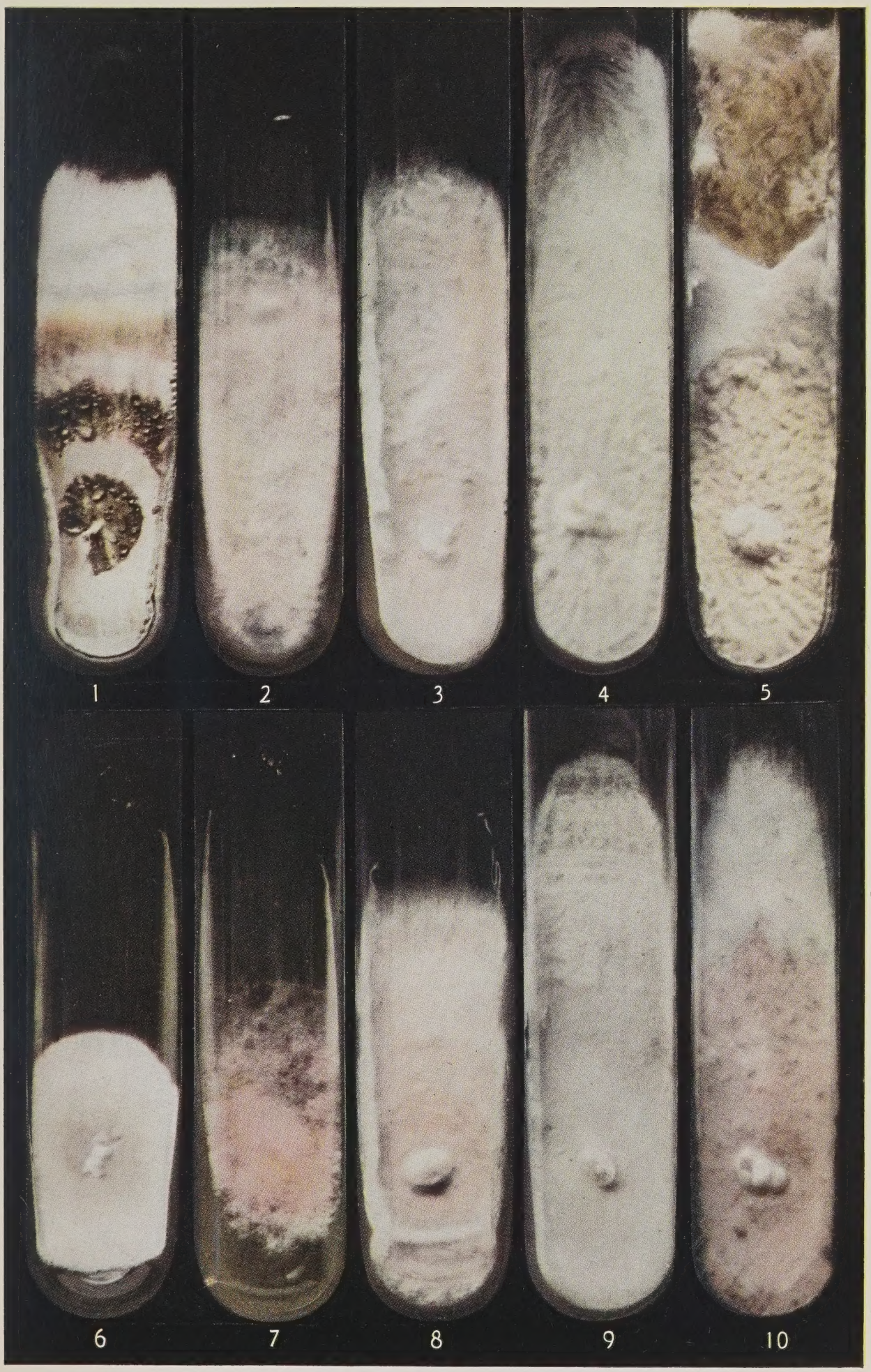
Fig. 98. A common method of branching in hyphae from aerial mat on potato-dextrose agar.

PLATE XII

Polyporus sulphureus

Fig. 99. Terminal chlamydospores from aerial mat on potato-dextrose agar.
Fig. 100 (*a-d*). Intercalary chlamydospores from aerial mat on potato-dextrose agar.
Fig. 101. Chlamydospores from aerial mat on corn agar.
Fig. 102 (*a-d*). Chlamydospores from submerged mycelium on potato-dextrose agar.
Figs. 103 and 104. Hyphae of first formed type from potato-dextrose agar culture.
Fig. 105. Fibre-like hypha from aerial mat on potato-dextrose agar.
Fig. 106. Origin of fibre-like hypha from hypha of first formed type.

PLATE I.



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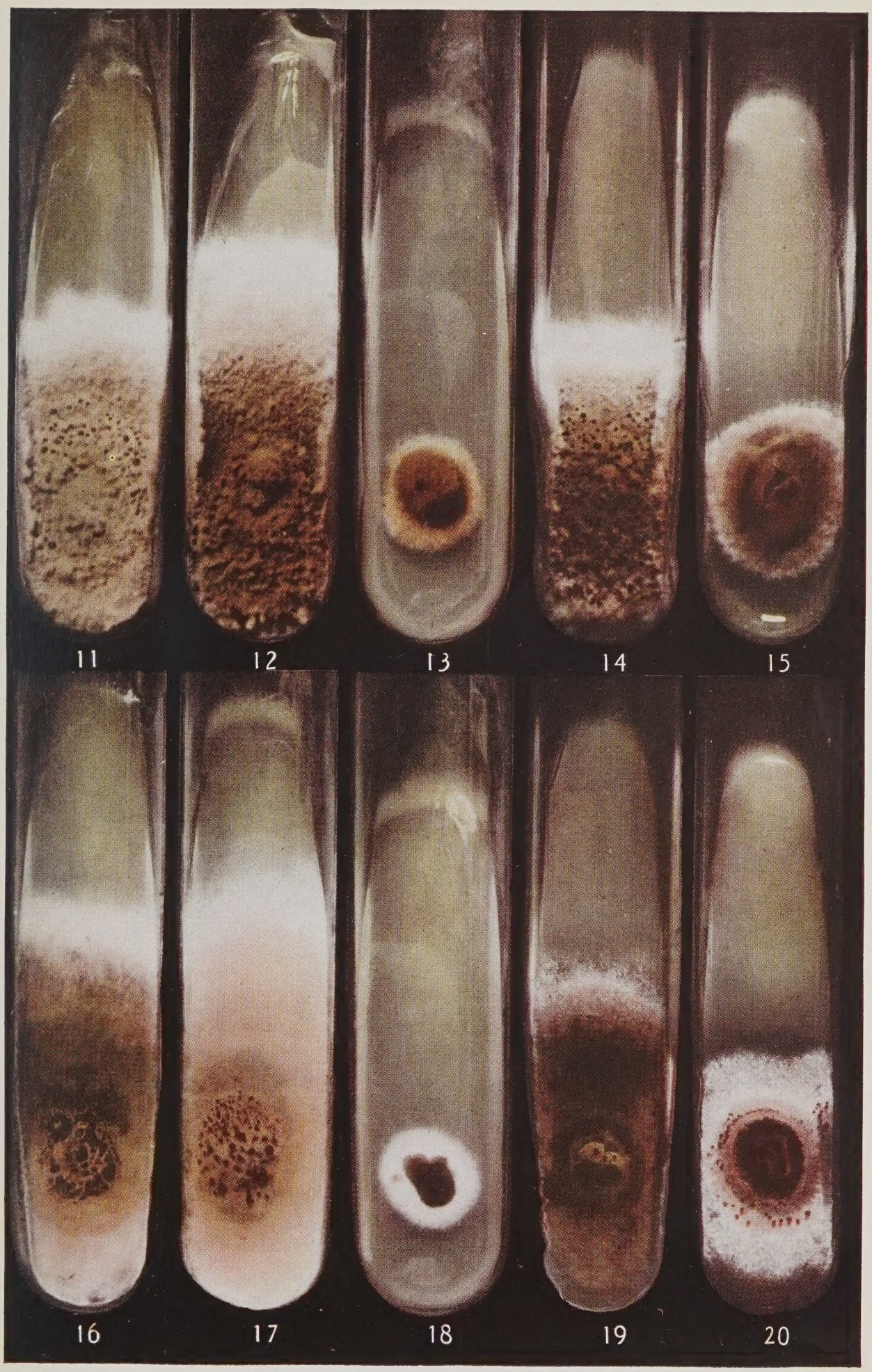

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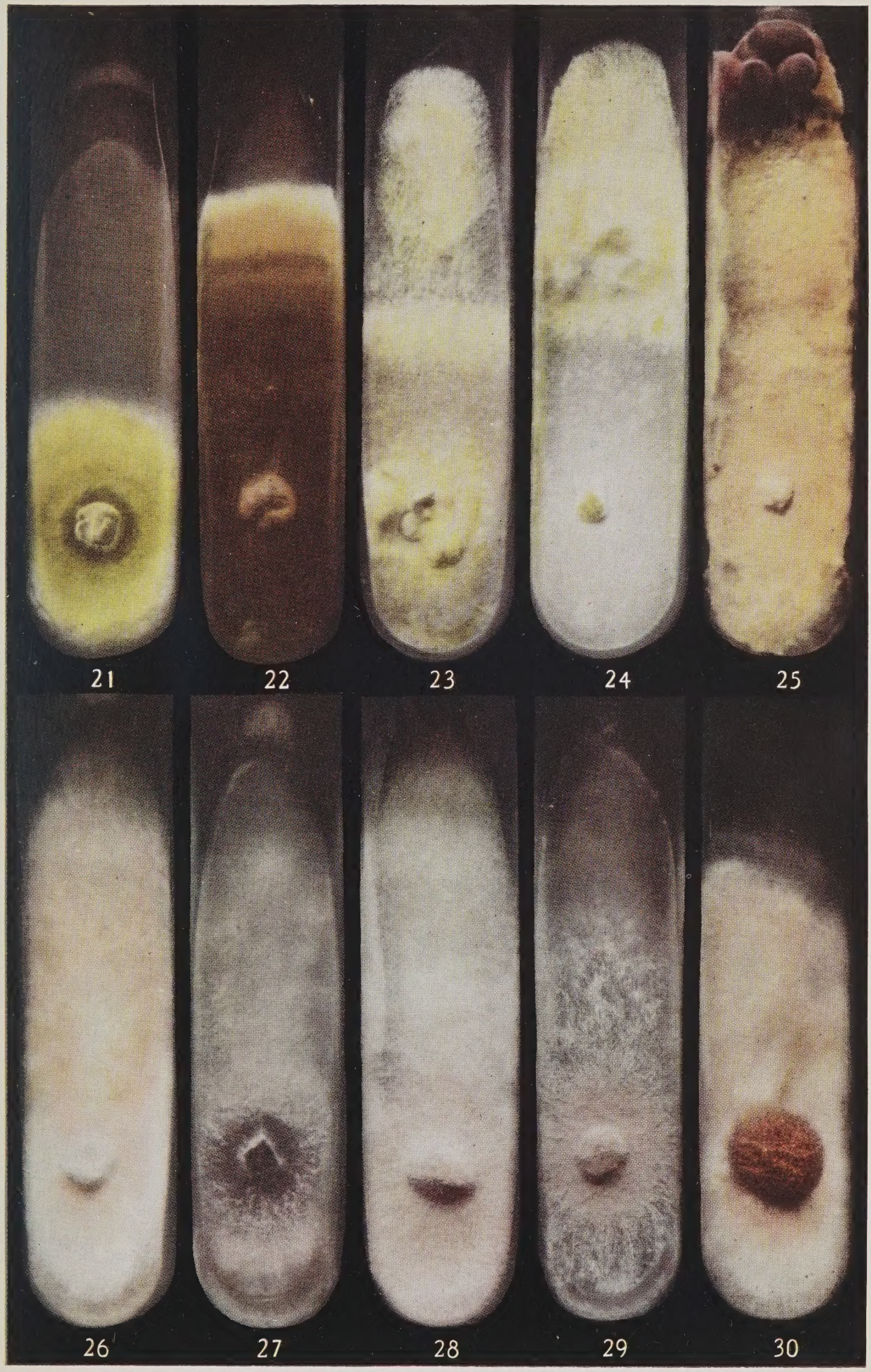

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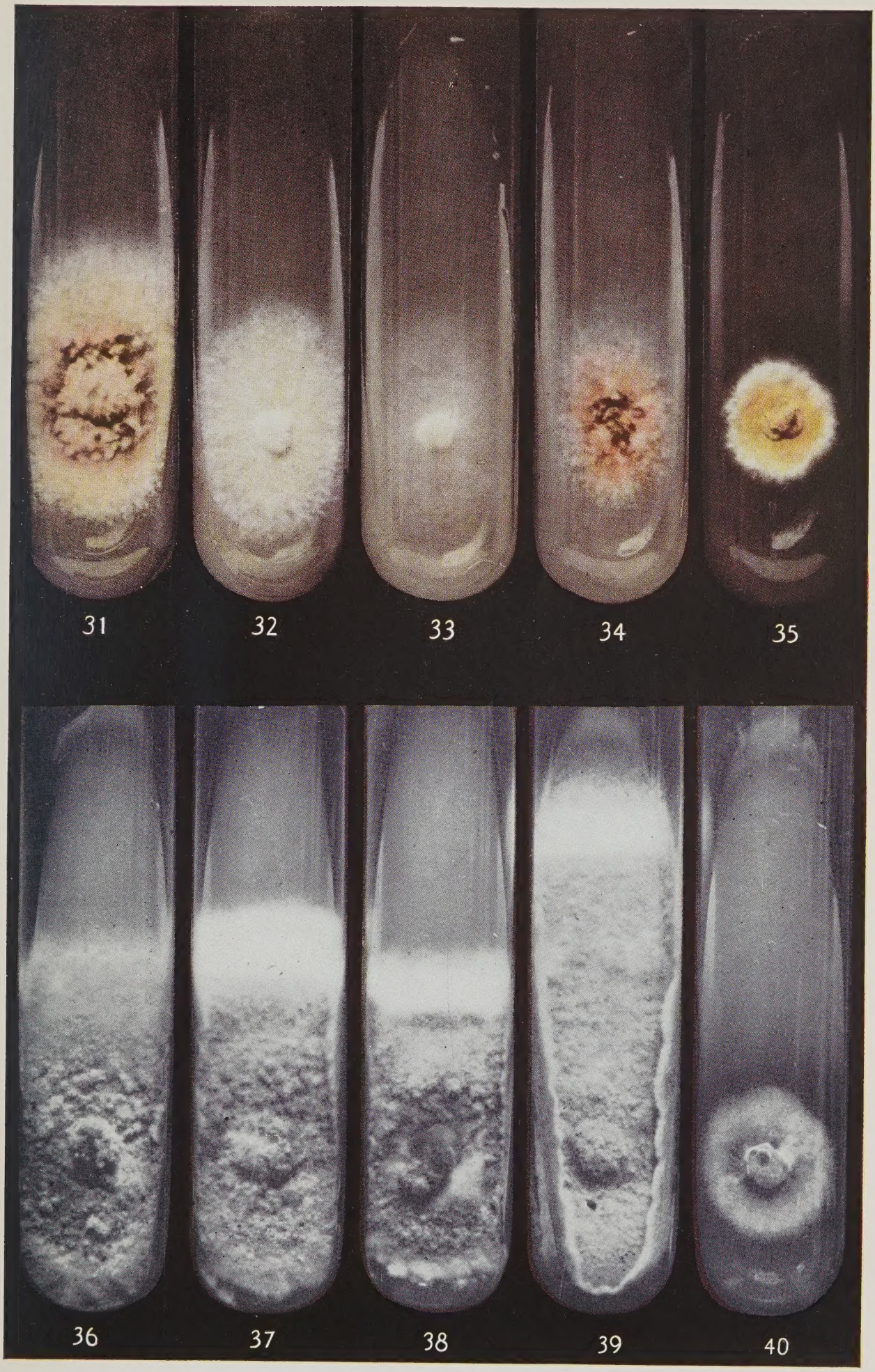

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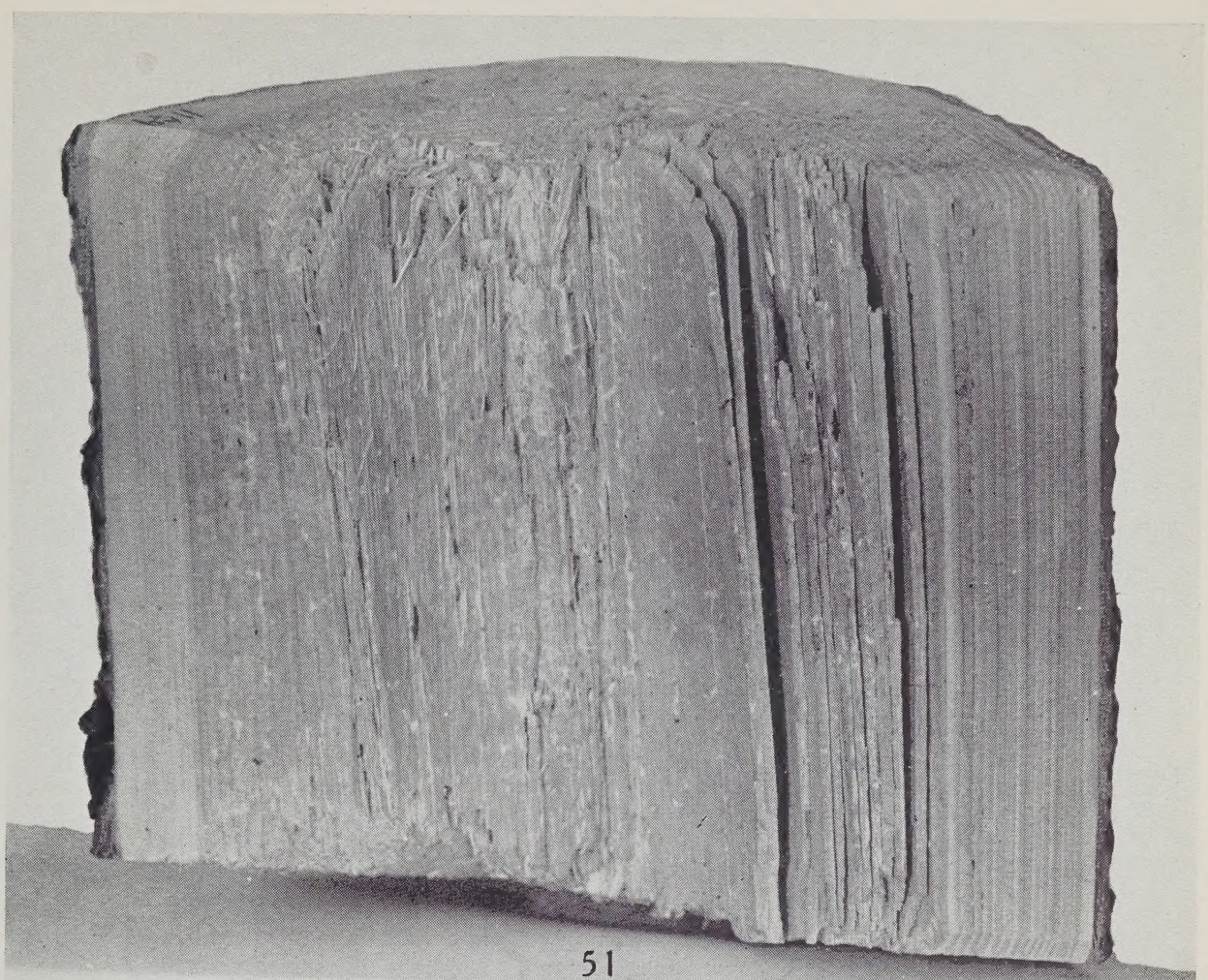

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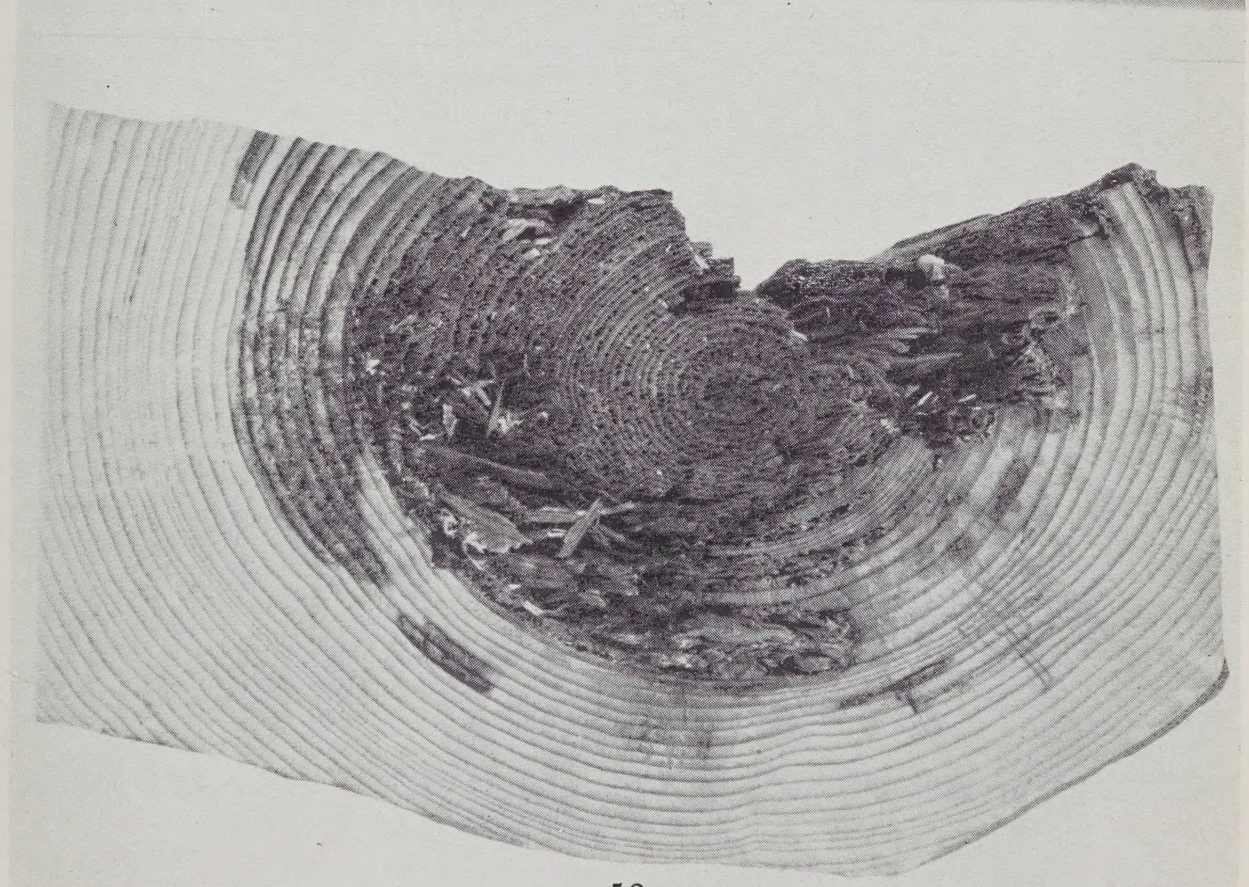

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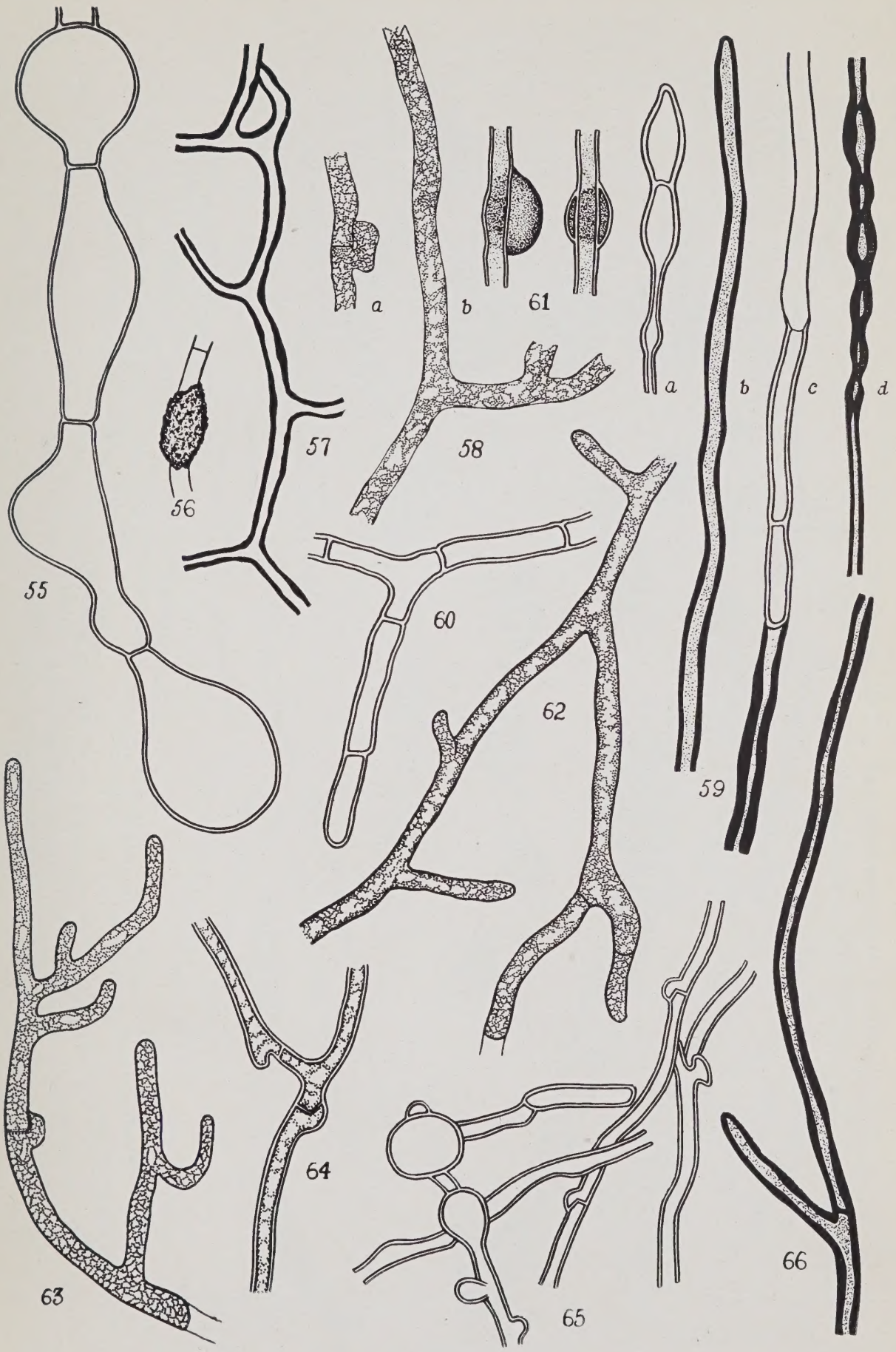

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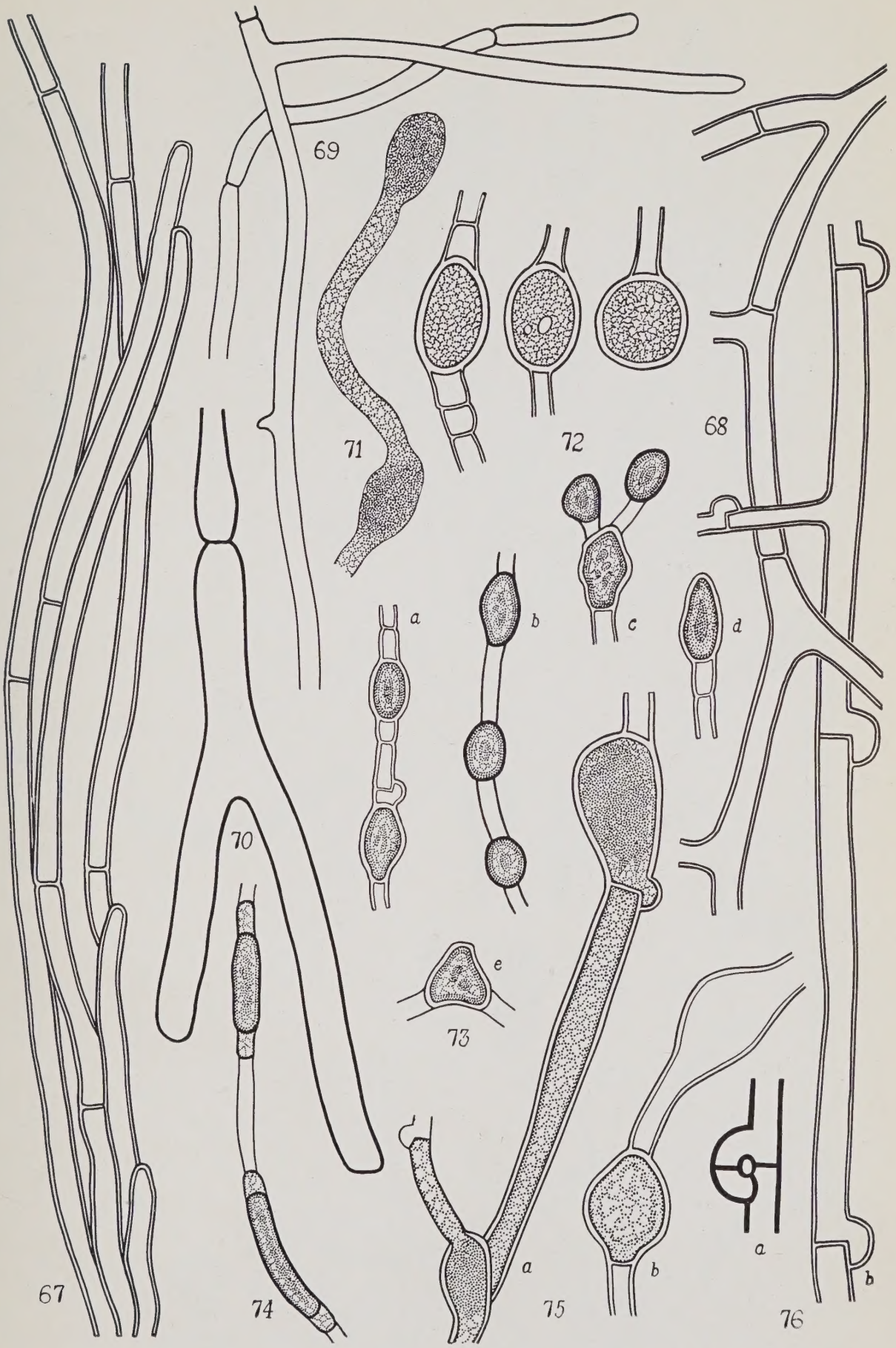

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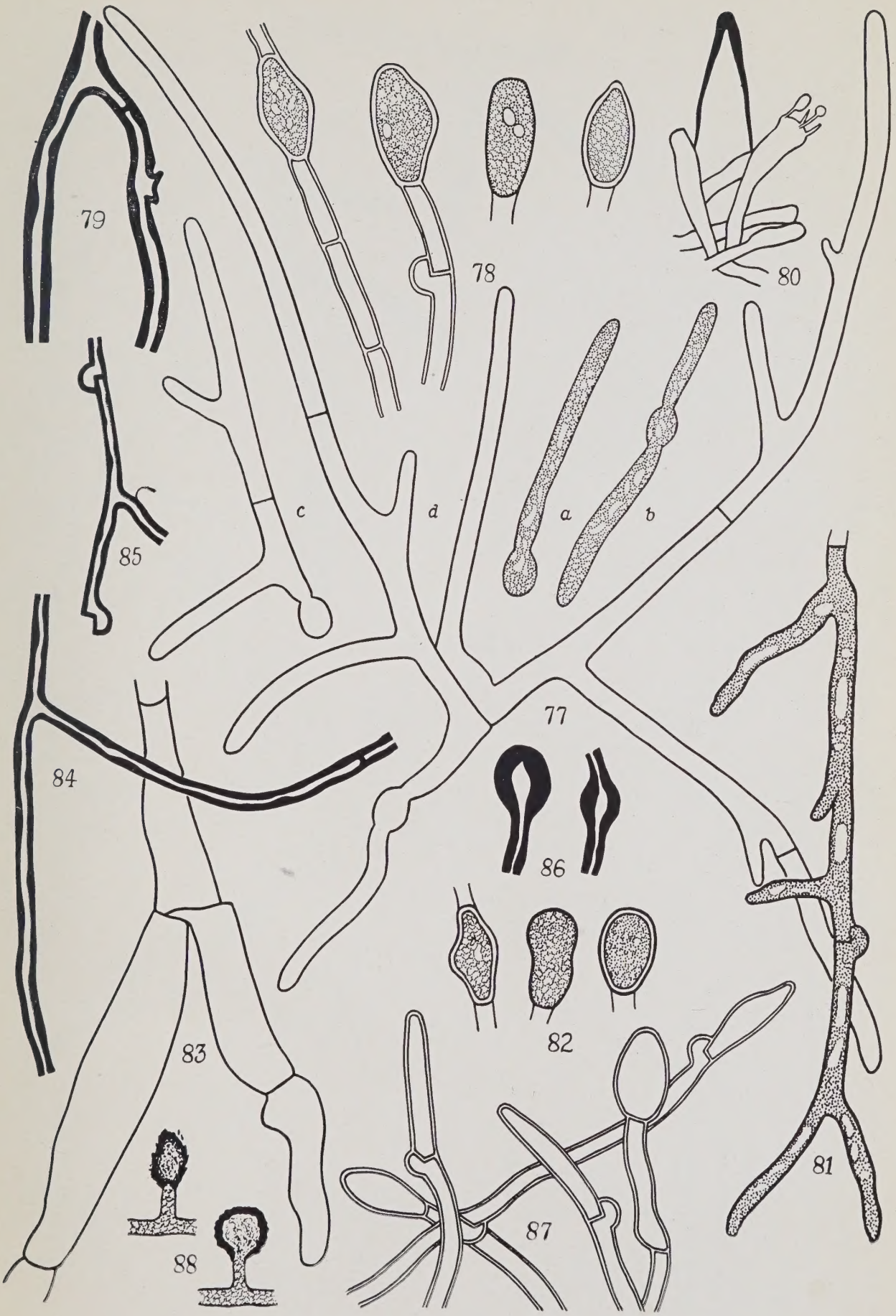

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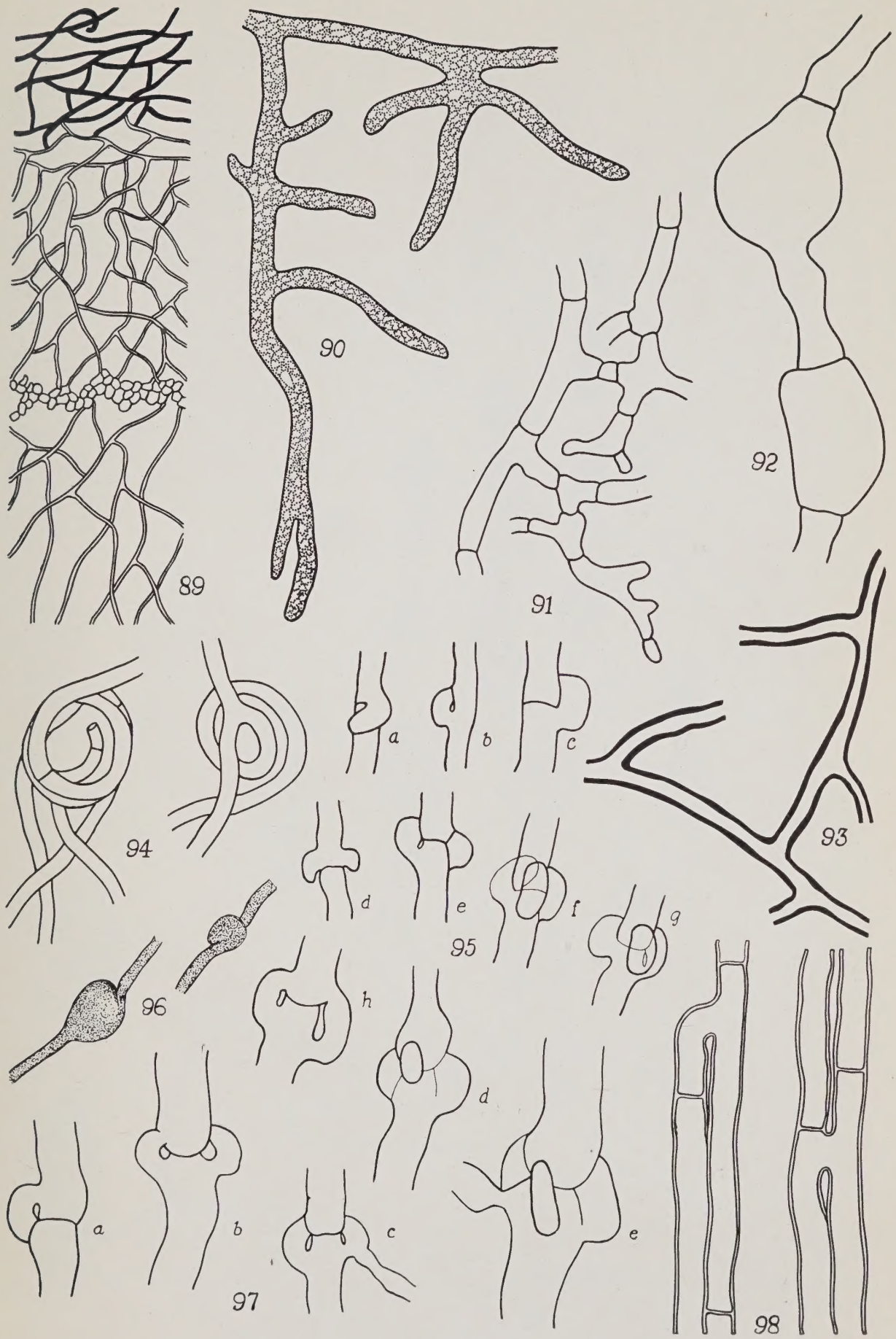

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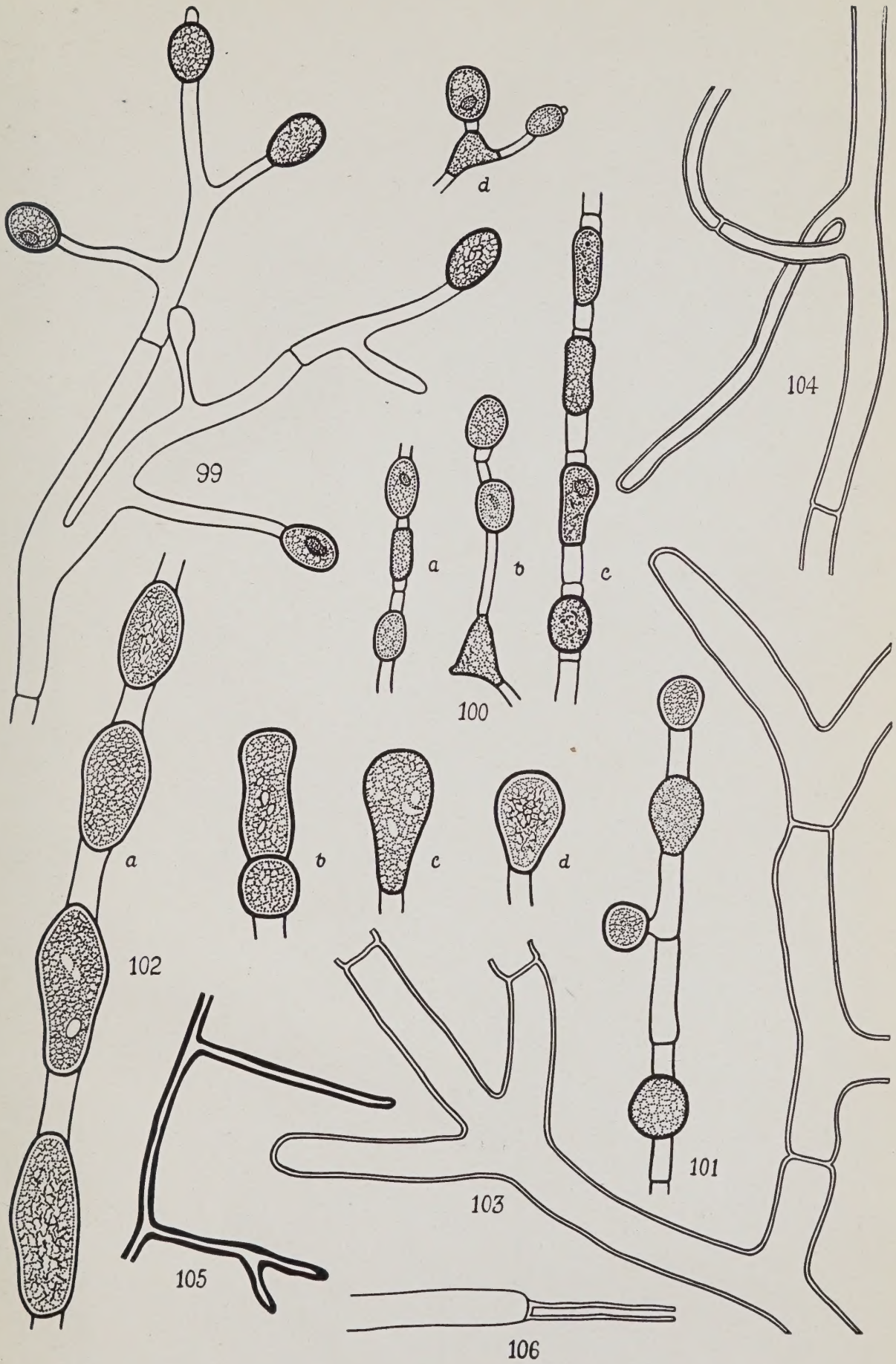

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